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SOME AFRICAN DIPTERA PUPIPARA.

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(With 6 Text-figures.)

FOR the material herein dealt with, I am indebted principally to Mr G. A. H. Bedford of the Division of Veterinary Services of South Africa. Other material collected by the writer from bats in the United States National Museum some years ago and a specimen received from Dr C. R. Kellogg of Foochow, China, are also recorded.

In this paper, as in others by the writer, the policy is followed of describing as new everything that cannot definitely be connected with named forms.

Family HIPPOBOSCIDAE.

Genus **Ornithomyia** Latreille.

Three species referred to this genus have been recorded from South Africa. The species of this group, however, are very much confused and positive identifications are difficult, if not impossible. I am therefore describing a species at hand from South Africa as new. This species raises a question concerning the validity of the genus *Pseudornithomyia* of Lutz, Neiva and Costa Lima, a genus that is separated from *Ornithomyia* because of the absence of ocelli and is based upon a single South American species, *P. ambigua* L., N. and C. L. Of the two specimens at hand, one male and one female, the female possesses ocelli, although they are extremely minute, while in the male they are definitely lacking. On this basis these two specimens would go into separate genera, but they seem definitely to belong to the same species. As far as can be determined from the description and figures of *P. ambigua*, this and the species at hand are very similar. The suspicion that the genus *Pseudornithomyia* cannot be maintained seems justifiable.

Ornithomyia inocellata n.sp.

Figs. 1, 2.

Material examined. Holotype, a female, and allotype, from *Petrochelidon spilodera*, a cliff swallow, Anderstepoort, Pretoria, South Africa (Bedford).

Female (Fig. 1). Length, on slide, 5 mm.; length of wing 5·5 mm. A pale species, as represented by the material at hand, but this probably somewhat teneral. Distinguishable from specimens identified as *O. avicularia* (Linnaeus)

and *O. lagopodis* Sharp, and from *O. fringillina* Curtis as described by Massonat, chiefly by the characteristics of wings and abdomen. Head (Fig. 2 B) of the type normal for the genus, but with the ocelli extremely small. Thorax with the normal pattern of setae, but with these somewhat more numerous than in the other species mentioned, the scutellum with a rather conspicuous

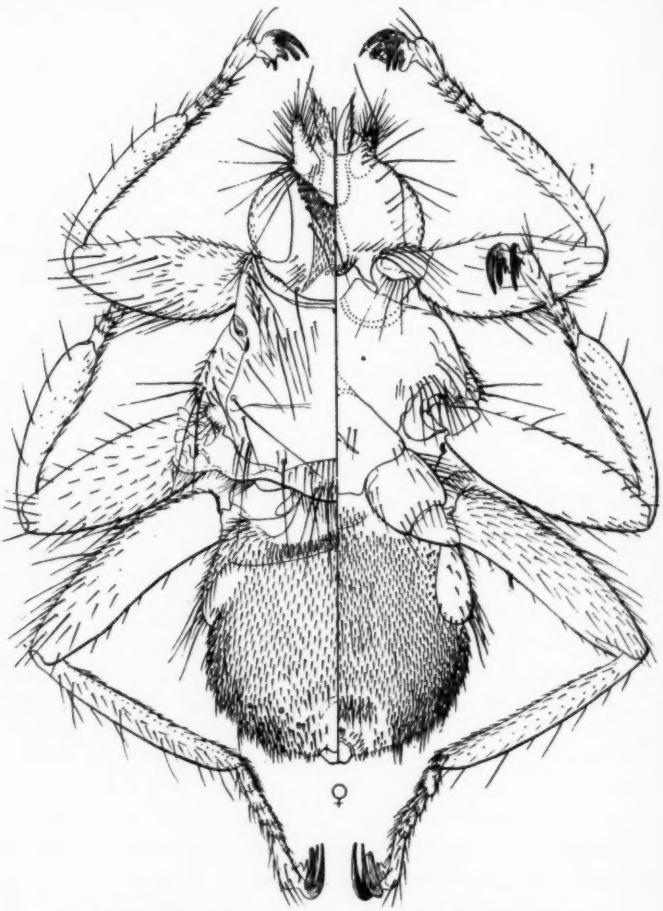

Fig. 1. *Ornithomyia inocellata* n.sp. Female, wings removed.

apical tuft. Pleurotergites somewhat swollen. Posterior tarsi (Fig. 2 D) with a very distinct, transverse comb of setae at the base of the first segment. Wings (Fig. 2 A) with the typical venation, the veins (in these specimens) quite pale; "second cross vein" almost behind the first; "third cross vein" strongly diagonal; setulae present uniformly on both sides of the wing from just distad of the first and second cross veins to the apex; wings slightly

fumate except for the basal portion from the costa to the posterior border of the anal cell and from the base to just distad of the first and second cross veins. Abdomen entirely without sclerotic areas, other than the basal plate, which is strongly produced posteriorly at the lateral angles and extends somewhat to the ventral side of the body. Basal plate, except for the lateral lobes, and the remainder of the abdomen thickly beset with setae, both dorsally and ventrally, except for a small apical area on either side, these setae being somewhat larger and longer at the lateral margins.

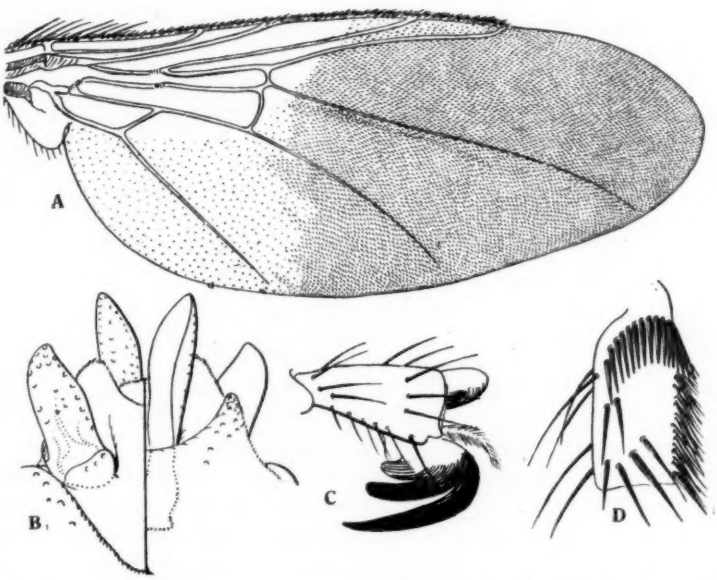

Fig. 2. *Ornithomyia inocellata* n.sp. A, wing, to same scale as Fig. 1; B, anterior region of head; C, terminal tarsal segment and claws; D, first tarsal segment of posterior legs, showing comb of setae.

Male. In size, general form and appearance practically identical with the female but entirely without ocelli. Abdomen with no tergal plates other than the basal and with setae even more numerous than in the female and more slender and longer. Claspers very small, the genital opening fringed with long setae.

Notes. It is, of course, conceivable that these two specimens belong to different species, but their close general similarity and their occurrence upon the same host seem to preclude this. The ocelli in the female may be regarded as vestigial.

Genus **Echestypus** Speiser.

This genus is evidently very close to *Lipoptena*, but the absence of the ocelli and the great reduction, or perhaps even total absence, of the palpi, offer bases for the technical separation of the two groups. As far as known it is purely African. Specimens at hand seem to belong to one of the five named forms.

Echestypus paradoxus (Newstead).

Figs. 3, 4.

1907. *Lipoptena paradoxa* Newstead, *Annals of Tropical Medicine and Parasitology*, **1**, 91–93, tf. 19, 20.

1927. *Echestypus paradoxus* (Newstead), Bedford, *Report Director Veterinary Education and Research, South Africa*, **11–12**, 782.

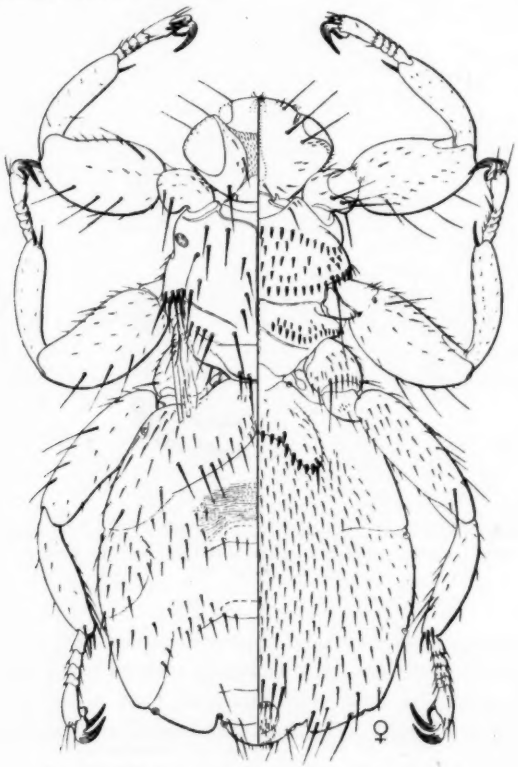

Fig. 3. *Echestypus paradoxus* (Newstead). Female.

Notes. Specimens at hand from *Nyala angasi* and *Sylviacapra grimmi* have been received through the kindness of Mr Bedford and are part of material previously recorded by him. I take the opportunity to present figures and notes which will supplement the existing literature.

The palpi, while extremely small and in some specimens retracted into the

head, are clearly recognisable (Fig. 4 B) and apparently constitute the "minute truncated cone" referred to by Newstead. The antennae are of the same type as those of *Lipoptena*, which have been well described by Jobling. Between the antennae and the eyes there is a deep, slit-like depression in the side of the head. The thorax is beset ventrally with rows of almost tubercle-like setae not noted by Newstead. The last tarsal segment, with the pulvilli and the empodium, is shown in Fig. 4 C.

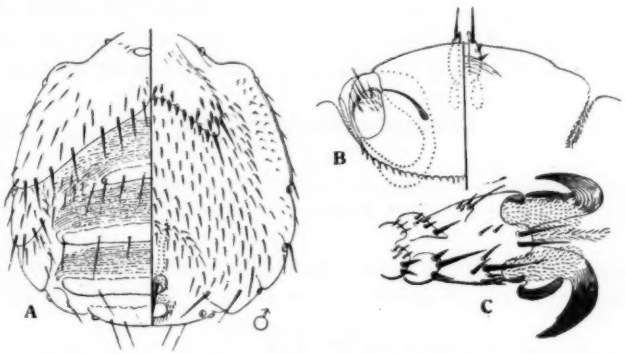

Fig. 4. *Echestypus paradoxus* (Newstead). A, abdomen of male, to somewhat smaller scale than Fig. 3; B, anterior region of head; C, terminal tarsal segments and claws.

The abdomen is rather strongly sexually dimorphic, being much shorter and relatively broader and with the apex strongly curved downward in the male. Also in the male, the median dorsal plates are relatively large and distinct as compared with those in the female and there is an extensive area of irregular, transverse striations which are but faintly indicated in the female. The first abdominal sternite is strongly arcuate, with the posterior margin beset with short, stout setae. The claspers of the male are obsolete and the genitalic structures are very small. It is not possible to describe them in detail from the specimens at hand.

Family STREBLIDAE.

Genus **Raymondia** Frauenfeld.

This genus is represented in the material at hand by two quite distinct species, one of which is apparently new. Although undoubtedly closely related to *Nycteribosca*, the genus presents a characteristic facies, the head broad and flattened, eyeless, with the antennae born in open pits, the abdomen with two longitudinal crests of large setae similar to those in *Nycteribosca*. The wings are very similar to those of *Nycteribosca* and are beset with very minute setulae.

The males are very similar to the females, except that the abdomen terminates in a conical portion of greater or less length, which is somewhat more sclerotic than the rest of the abdomen. The abdomen of the male is strongly curved downward, bringing the genital opening beneath the body.

The external genitalia are represented merely by minute vestiges of the claspers. The intromittent organ is extraordinarily long and slender, terminating in a somewhat expanded bulb that is beset with spines. There are slight differences in these structures in the males of the two species at hand, but they are scarcely available for specific differentiation.

The spiracles are exceedingly small. Even the mesothoracic spiracle, which in most of the Diptera Pupipara is of rather large size, is difficult to detect. I have been able to find but four abdominal spiracles. A similar condition exists in the genus *Nycteribosca*. The halteres (Fig. 6 F) are quite large and flattened. The large setae of the body (Fig. 6 D) are spirally marked.

Raymondia pagodarum Speiser.

Fig. 6 C.

1900. *Raymondia pagodarum* Speiser, *Archiv für Naturgeschichte*, **66** (1), 50, tf. 5.

1925. *Raymondia pagodarum* Speiser, Kessel, *Journal New York Entomological Society*, **33**, 24, f. 18.

Previous records. Recorded by Speiser from undetermined bat, Mahamaleipur, Madras, India, and by Kessel from *Hipposideros speoris speoris*,

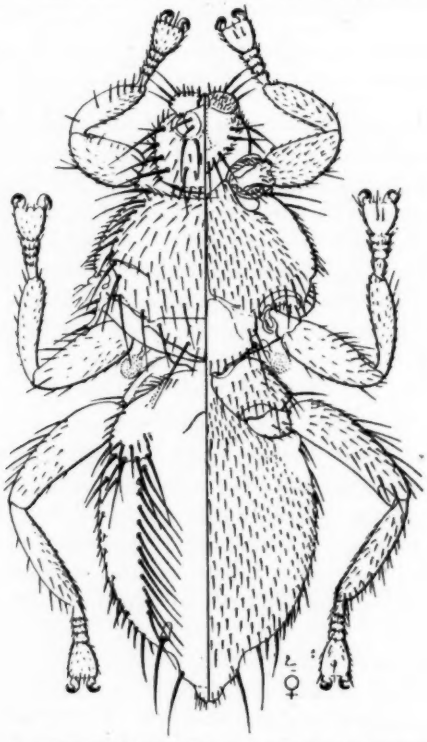

Fig. 5. *Raymondia bedfordi* n.sp. Female, wings removed.

Ceylon; *H. larvatus* (or *Rhinolophus malayanus*), Malay Peninsula; *Emballonura nigrescens*, Molucca Islands and Amboina; and ? *Rhinolophus affinis*, Ceylon.

Present records. A male from *Hipposideros ruber*, Gondokoro, Uganda (U.S.N.M.), and a male from undetermined bat, Foochow, China (C. R. Kellogg).

Notes. On the basis of the existing literature the two specimens at hand appear to be this species rather than *R. huberi* Frauenfeld. The species is described as entirely lacking the basal lobe of the wings, but this is in error. As may be noted in Fig. 6 C, this lobe is present although it is quite small and is without setae except for a single, quite large one at its apex.

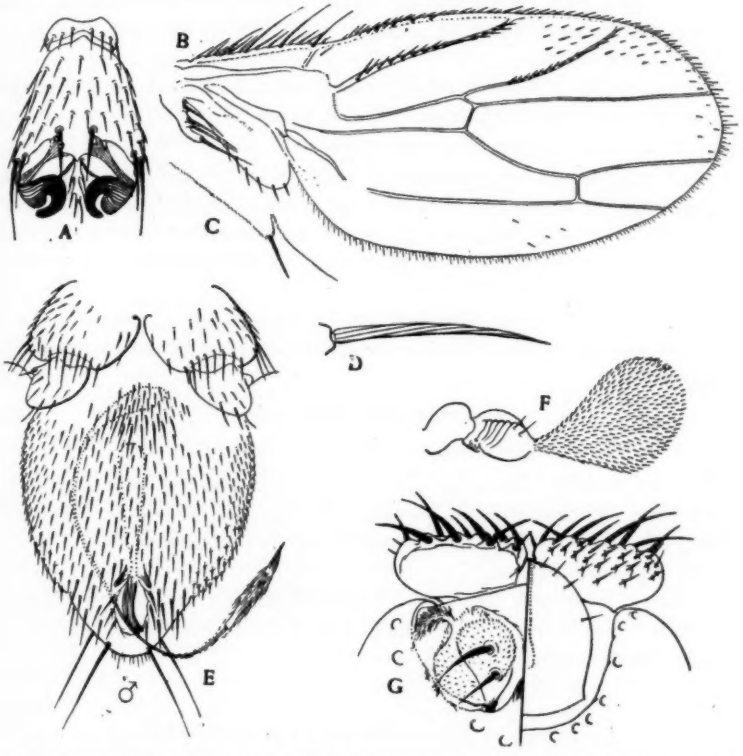

Fig. 6. *Raymondia bedfordi* n.sp. A, terminal tarsal segment and claws; B, wing, to same scale as Fig. 5; D, spirally marked seta; E, ventral aspect of abdomen of male, to somewhat larger scale than Fig. 5; F, haltere; G, anterior region of head. *Raymondia pagodarum* Speiser: C, basal lobe of wing.

Raymondia bedfordi n.sp.

Figs. 5, 6.

Material examined. Three females and one male from *Nycteris damarensis*, Kaokovela, South-west Africa (G. A. H. Bedford). Holotype, a female, and

allotype retained in the Stanford University collections, paratypes returned to Mr Bedford.

Female (Fig. 5). Length on slide 1·75 mm. Of the facies common to the genus and apparently nearest to *R. lobulata* Speiser. It agrees with the latter in the possession of a well-defined basal lobe on the wings (Fig. 6 B), this bearing a marginal row of small setae. It differs from *R. lobulata* as described and figured by Speiser in having the sternum of the mesothorax quite uniformly covered with small setae, while of *R. lobulata* Speiser has said: "Ausser dem Vorderrand sind nur die beiden äusseren Viertel des Mesosternum beborstet, während auf den inneren beiden nur je eine einfache Linie kurzer, nach hinten und innen weisender Börstchen jederseits neben der Medialfurche steht. Zwischen dieser Börstchenreihe und den seitlichen beborsteten Bezirken ist eine ganz kahle Fläche."

The wings (Fig. 6 B) are beset almost throughout with minute points which can scarcely be shown in the figure. The abdomen bears dorsally a somewhat indistinct basal plate with numerous quite long setae on its lateral lobes, and also the characteristic two longitudinal crests of long, stout setae. What may be regarded as the apical seta of each of these rows arises from a small, sclerotic area. Otherwise the dorsum is bare except for the extreme margins. Venter with numerous small setae, intermingled with some larger setae near the lateral margins.

Male. Length 1·5 mm.; similar to the female in general form except for the downward curved abdomen which terminates in a short, somewhat conical, end segment.

Genus **Nycteribosca** Speiser.

Nycteribosca kollari (Frauenfeld).

Previous records. Recorded by Falcoz and Speiser from *Rhinolophus hipposideros, R. mehelyi, R. euryale, Miniopterus schreibers, Phyllorhina tridens, Rhinopoma microphyllum* and *Vespertilio murinus*, from Sardinia, Algeria, Tunis and Egypt.

Specimens examined. Two males from *Rhinolophus mehelyi*, Province Sassari, Italy (U.S.N.M.), and a male and female from *R. geoffroyi*, near Pretoria, South Africa (Bedford).

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A REVISION OF THE GENUS *RAYMONDIA* FRAUENFELD (DIPTERA PUPIPARA, STREBLIDAE).

By B. JOBLING.

From the Wellcome Bureau of Scientific Research, London.

(With 10 Text-figures.)

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Introduction.

In the course of my previous work on the structure of the head and mouth parts of the Streblidae (1929), I encountered some difficulty in identifying the different species of the genus *Raymondia*. A careful examination of the specimens in the collection of the British Museum and of my own specimens showed that the descriptions and figures given by the authors who studied these insects in the dried condition are not quite adequate. In dried specimens of these minute flies it is often impossible to distinguish clearly the chaetotaxy of the head and the thorax and the characters present in the wings, which are generally interlocked. Moreover, the parts of the male genitalia lying in the abdomen are absolutely inaccessible. When examined in the cleared specimens the genitalia of the different species have been found to possess distinctive characters.

This paper is a redescription of the three known species and a description of two new ones from specimens mounted in Canada balsam. All the figures illustrating this paper were made with the aid of a camera lucida.

I am indebted to Mr F. W. Edwards, of the British Museum, Natural History Department, for placing at my disposal the specimens of the genus *Raymondia* in the collection of that institution.

Technique.

Most collectors preserve ectoparasitic insects in 70 per cent. alcohol; this method is quite efficient. Some, however, preserve them dry by gluing or pinning them to a card—an old-fashioned method most unsatisfactory for the study of these insects. The following methods, which were found to be most suitable, were used in the present investigation. The Streblidae preserved by one of the above methods are cleared and mounted on a slide in Canada balsam. In alcohol-preserved specimens the wings are detached with fine forceps as close to the thorax as possible, unfolded, and, after being cleared in oil of cloves, mounted on a slide in Canada balsam. The wingless body is then transferred to a 10 per cent. solution of caustic potash, where it is left to soak for about two days and then boiled. It was found that boiling alone, without previous soaking, does not dissolve the contents of the body so well in specimens preserved for a very long time in spirit. When the soft parts have been liquefied and the specimen is clear, it is transferred to water. In this medium the legs are straightened with two dissecting needles, care being taken to avoid distortion of the body. When the legs are set in the desired position the specimen is transferred first to 95 per cent. and then to absolute alcohol, cleared in oil of cloves and mounted in Canada balsam on the same slide with the wings. It is essential to support the coverslip with three pieces of glass, otherwise the specimens are liable to be distorted by pressure and rendered useless for study. With dried specimens the manipulation is slightly different. The specimen is first detached from the card by immersion in water and will generally be found floating dry on the surface after the glue is dissolved. It is then transferred for a few seconds to 95 per cent. alcohol to facilitate further soaking, and then removed to a solution of caustic potash and slightly heated. During the heating the wings become softened, while the expanding air in the veins unfolds them. As soon as the wings unfold the specimen is transferred to water; the wings are then detached and mounted on the slide. The wingless body is returned to the potash and boiled until all the air bubbles have disappeared. The further manipulation is the same as in the case of spirit-preserved specimens. Pinned specimens must be slightly softened in potash before the pin is removed and the wings are separated from the body.

General morphology of the genus *Raymondia*.

In the Streblidae in general the colour is of very little taxonomic value, and has practically no significance for the differentiation of species of the genus in question. Consequently the identification is chiefly based on their morphology, which, with the exception of the head (Jobling, 1929), has been very little studied. This section is devoted to a brief discussion of the morphological characters which are of importance for the separation of the species.

In all members of the genus *Raymondia* eyes are absent. In some species the head is rounded when viewed dorsally and is as broad as the anterior part of the thorax, while in others it is more or less quadrangular. Its dorsal and

ventral surfaces are flat. The anterior part of the dorsal surface is occupied by the fronto-clypeus, which bears the antennae and is attached by the membrane to the genae laterally and to the vertex posteriorly. The vertex occupies the major portion of the dorsal surface of the head and is subdivided into several anatomically distinct subregions as follows (Fig. 1): The lateral setose areas are the latero-vertices; anteriorly each of them passes into the gena and laterally into the postgena. The narrow membranous strip extending from the fronto-clypeus between the latero-vertices is the medio-vertex. This corresponds to the frontalia of other authors. The most posterior part of the vertex is the postvertex; this corresponds to the vertical triangle. The postvertex extends to the posterior border of the head, where it passes into the occiput. The occiput occupies half of the posterior surface of the head, extending ventrally to the occipital foramen. The regions lying ventral to the occipital foramen represent the postgenae; they also occupy all the ventral surface of the head except the most anterior part, where each of them passes into the gena. Together with the occiput the postgenae form very deep depressions on each side of the occipital foramen for the coxae of the anterior legs. In the middle of the ventral surface of the head the postgenae are separated from each other by the membranous space; this is stippled in all the figures showing the ventral aspect of the head. In the anterior part of this membrane is attached the labium, which is formed externally by the theca (*t.*) and a small conical structure representing the greatly modified labella (Fig. 2 B, *lb.*). The palps are small and very flat, they project between the labium and the fronto-clypeus antero-dorsally.

The thorax in this genus is ovoid or rounded when viewed dorsally. Its dorsal surface is only slightly convex, while the ventral is perfectly flat. The prescutum occupies the major portion of the mesonotum. In *R. pagodarum* it is completely separated by the mesonotal suture from the scutum (Fig. 5); in *R. quadriceps* this suture is not quite distinct in the middle (Fig. 7), while in all the other species it is incomplete. The more or less triangular scutellum is sharply delimited from the scutum by the suture. The humeral processes are very distinct and bear one or two very strong and several small setae. They are very prominent in *R. huberi*, moderately large in *R. lobulata*, *R. planiceps* and *R. pagodarum*, but very small in *R. quadriceps*. In all the species there is present one very long and strong posthumeral seta.

The largest part of the ventral surface of the thorax is occupied by the sternoplevrae of the mesothorax. Each of these is separated from the two sclerites which occupy the most posterior part of the ventral surface of the thorax by the transverse suture between the coxae of the middle legs. The greater portion of these sclerites is formed by the plevrotrochantins of the metathorax, since the furcasternae are crowded posteriorly and form only the posterior edge of the plevrotrochantins. The legs are strong, the hind pair being slightly elongated. The last segment of the tarsus is considerably enlarged. The front coxae are widely separated.

The wings are short and broad, with five longitudinal and two cross veins. The costa (Fig. 2 A) is very setose in all species; in *R. lobulata*, *R. planiceps* and *R. quadriceps* it extends along the margin of the wing as far as the fourth longitudinal vein (*M.* 1 + 2), but in the two species last named it is very faint between the third (*R.* 4 + 5) and the fourth longitudinal veins; in *R. pagodarum* and *R. huberi* it is already not quite distinct between the second (*R.* 2 + 3) and the third longitudinal veins. The subcostal vein is absent; the vein so described by different authors is really the humeral vein (*h.*). The first longitudinal vein (*R.* 1) is the thickest and very setose; together with the costa it encloses the costal cell. The second longitudinal vein is thin and separates the marginal cell (1 *R.*) from the submarginal (3 *R.*). The first posterior cell (5 *R.*) is separated from the first basal cell by the anterior cross vein (*r.m.*). The second posterior cell (2 *M.* 2) is separated by the posterior cross vein (*M.* 3 + 4) from the discal cell (1 *M.* 2), which is fused with the second basal cell. The third posterior cell (*Cu.* 1) is formed by the fifth longitudinal vein (*M.* 4 + *Cu.*1) and the inner margin of the wings. The inner margin is fringed with very fine long and short microtrichia. The fifth longitudinal vein does not reach the wing margin in every species. In some species there is a vestige of the second anal vein (2 *A.*). In the wings of *R. lobulata* (Fig. 2 A) this vein completely separates the alula (*al.*) from the rest of the wing. The alula is present in all the species, it is well developed and fringed with several setae in *R. lobulata* and *R. quadriceps*, but it is vestigial and has only one terminal seta in the other species. Arising from the posterior part of the base of the wings is the calypteron (*ca.*), which represents the modified squama. The halteres are ovoid and moderately large.

The abdomen of the female is sacciform; its shape, however, is largely determined by the size of the larva developing in the uterus and the amount of blood taken during feeding. The abdomen ends in an apical cone which is separated from the main portion by a membranous fold. The basal tergite-like part forms a large lobe on each side of the dorsal surface. This lobe bears several strong setae, those arising from the posterior margin of the lobe being the longest abdominal setae. In the extreme antero-lateral part of the abdomen on each side of the narrow base there is another lobe-like elevation, it is very small and bears a few short setae. The dorsum of the abdomen has two longitudinal rows of very strong and long setae directed postero-laterally, and lateral to these are several strong but shorter setae. The surface of the dorsum between these rows and from the base to the apical cone is naked. The ventral surface is covered with short setae which gradually become much smaller and denser in the antero-lateral parts of the abdomen. In some species the transition of the setae is very abrupt. The basal sternite-like portion of the abdomen is slightly chitinised in its posterior part and covered with longer setae along the margin. In the male of *R. quadriceps* the sternite-like portion forms a narrow, weakly chitinised plate (Fig. 8 A).

The abdomen of the male differs from that of the female in the following

respects: it ends in a large slightly chitinised cone which is separated by a membranous fold from the main portion; the sides of the cone are covered with moderately strong setae. At the base of the cone on its ventral surface there is a small chitinous plate bearing two finger-shaped processes (Figs. 2, 10 C, *f.p.*). Posterior to this plate and not far from the apex of the cone is an orifice through which parts of the genitalia protrude.

I am restricting myself here to a general description of the genitalia, without discussing the homology of the different parts. There is a middle part, which is gutter-shaped and strongly chitinised towards the apex and ends in two claspers (*cl.*); one of these is generally much longer and darker in colour than the other. Both claspers protrude through the orifice. From the base of the middle part arises an apodeme (*ap.*); another apodeme is connected with the penis. These apodemes are shown in Fig. 10 B and C, by dotted lines. The penis consists of two tubular structures, one of which is enclosed within the other when the genitalia are at rest.

To conclude this section it is necessary to point out that the proportions and structure of the different parts of the head afford criteria for the separation of the species into two main groups: (1) Those species in which the head is rounded laterally and is as broad as the anterior part of the thorax, and the theca is twice as broad as long. The forms with these characters resemble each other in their general appearance (*R. lobulata* and *R. planiceps*). (2) Those species in which the head is more or less quadrangular and is narrower than the anterior part of the thorax, and the theca is as long as, or a little longer than broad (*R. pagodarum*, *R. quadriceps* and *R. huberi*). The characters exhibited by the other parts of the body are either limited to one species, or present in forms having either of the above-mentioned head characters.

KEY TO THE SPECIES OF *RAYMONDIA*.

1. Head as broad as anterior part of thorax; rounded laterally; theca twice as broad as long 2
   Head narrower than anterior part of thorax; more or less quadrangular; theca as long as or a little longer than broad 3
2. Alula well developed with several setae; each sternoplevra with an oblong, non-setose space (Fig. 2 B) ... ... **R. lobulata** Speiser.
   Alula vestigial with single terminal seta; sternoplevrae without non-setose space (Fig. 4 A) **R. planiceps** sp.n.
3. Mesonotal suture complete; fourth longitudinal vein strongly bent downwards after branching with anterior cross vein, first posterior cell enlarged in its basal part (Fig. 6 B) **R. pagodarum** Speiser.
   Mesonotal suture not quite distinct, or incomplete in the middle; fourth longitudinal vein nearly straight, first posterior cell enlarged at the posterior cross vein 4
4. Mesonotal suture not quite distinct; alula well developed with several setae (Figs. 7 and 8) **R. quadriceps** sp.n.
   Mesonotal suture incomplete; alula vestigial, with single terminal seta 5
5. Mesonotum sparsely covered with setae; these are about as long as the distance between them (Fig. 9)... ... ... **R. huberi huberi** Frauenfeld.
   Mesonotum densely covered with setae; these are about twice as long as the distance between them (Fig. 10 B) ... ... **R. huberi setosa** subsp.n.

RAYMONDIA Frauenfeld, 1855.

Strebla Kolenati 1856.

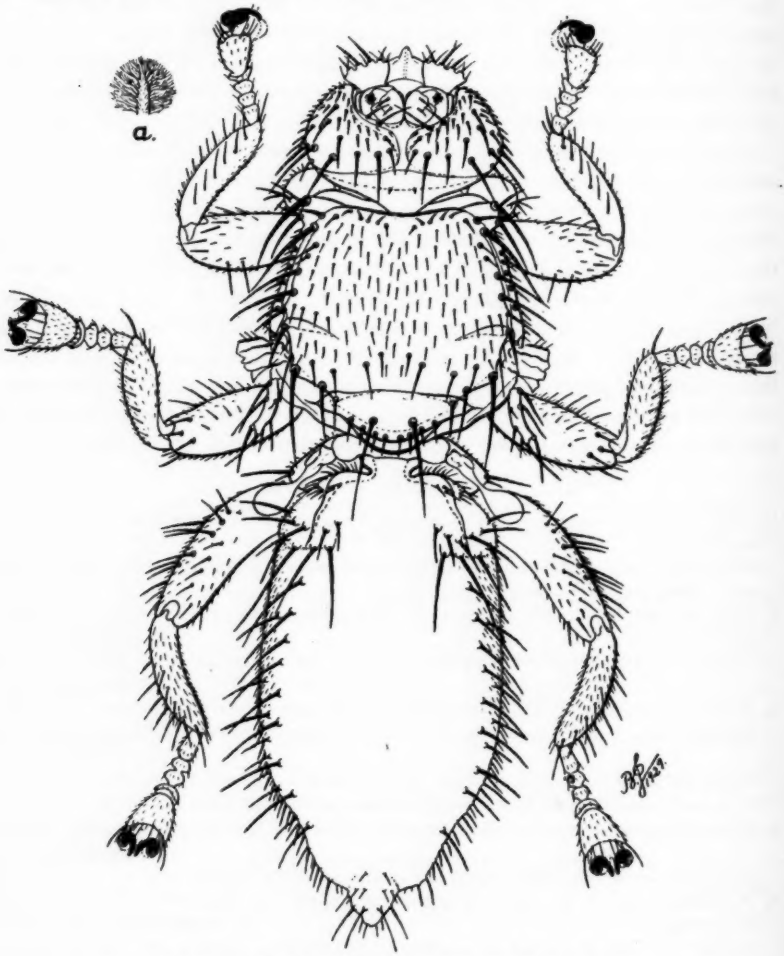
Raymondia lobulata Speiser, 1900.

Raymondia kollari Schiner, 1868 (*nec* Frauenfeld, 1855).

Previous record: off *Megaderma lira* from Ceylon; from bats in Madras.

Present record: about a hundred specimens off small brown bats, from Punjab, India.

Very many specimens were compared with the type received from the Vienna Museum, with which they agree in all details.



1. *Raymondia lobulata* Speiser, female. *a.* arista of antenna.

Female (Fig. 1), length (on slide) 1·9–2·3 mm.

Head as broad as the anterior part of the thorax, rounded laterally; subregions of the vertex clear, each latero-vertex chitinised along the anterior border, with small setae in the anterior and very strong, long ones in the

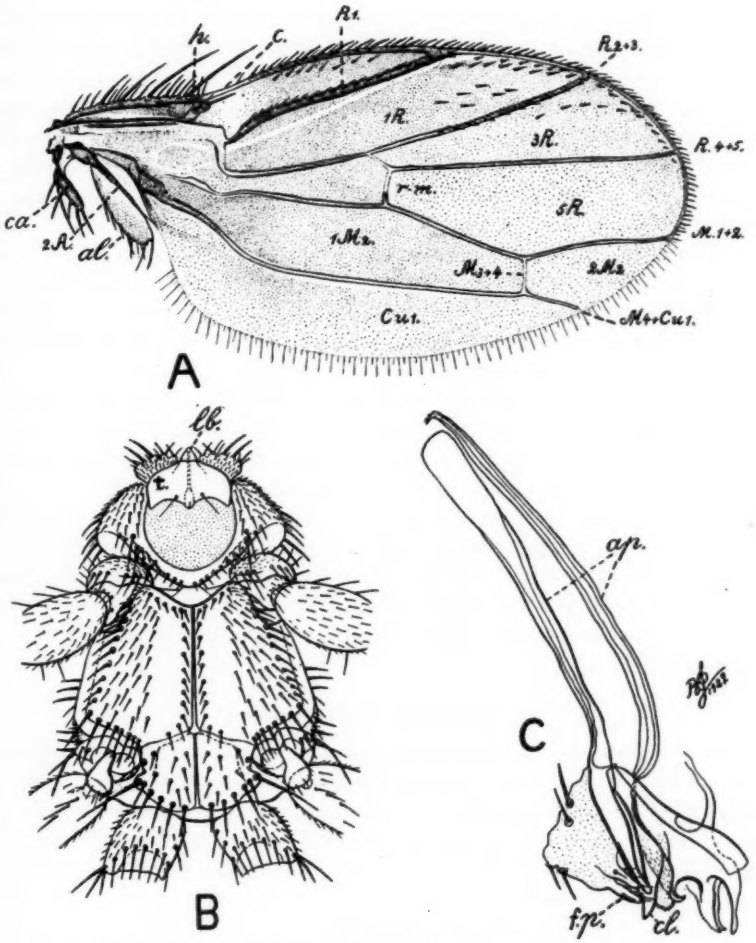


Fig. 2. *Raymondia lobulata* Speiser. A, wing; B, ventral view of head and thorax; C, genitalia of male.

posterior and postero-lateral parts; two or four very small but stout setae on the posterior margin of the vertex. A row of short setae on the postgenae along the posterior margin of the ventral surface of the head, a few long ones in front of the anterior coxae; the most anterior part of the postgena and the

genae bear short setae. Palps broader than long, with somewhat truncate anterior margin. Theca of the labium twice as broad as long.

Thorax with humeral processes moderately large, each with two long and a few very much shorter setae. Prescutum moderately covered with fine pale setae; a row of darkly coloured short setae at the anterior margin; one very strong posthumeral seta and a few strong ones along the lateral margin. Mesonotal suture incomplete in the middle. Scutum twice as short as the prescutum, sparsely covered with fine, pale setae and bearing several strong ones posteriorly. Scutellum broadly rounded, with two very long, strong setae closer to the posterior margin, which bears several short setae; a single seta in each lateral corner. Each sternoplevra with oblong, non-setose space separated by a single row of small setae from the median suture. Legs presenting no distinctive characters.

Wings (Fig. 2 A), 1·6 mm. long. Alula well developed with several setae; second anal vein very distinct, humeral vein with two setae; subcostal, marginal and submarginal cells with setae in the distal part, first posterior cell (5 *R.*) broad at the posterior cross vein (*M.* 3 + 4), fifth longitudinal vein gently bent in its basal part.

Abdomen ends in a small apical cone, which bears four long but comparatively thin setae; a few small setae on the dorsal and ventral surfaces of this cone. Setae of the ventral surface of the abdomen gradually pass into very small and much more numerous ones in the antero-lateral parts.

Male, 1·7 mm. long; closely resembles female in all respects except the apical cone, which is much longer and as broad at the base as long, its ventral surface is densely covered with fine short setae; laterally it bears four strong setae. Middle part of the genitalia broad but short, ending in two claspers which are short and nearly equal in length; the apodemes practically of equal length (Fig. 2 C).

Raymondia planiceps sp.n.

The holotype female, off bat from British East Africa. It was preserved in the collection of the British Museum (Natural History Department) under the name of *R. huberi*, apparently on account of the absence of well-developed alula. However, in general appearance, this form resembles *R. lobulata*, but differs from it in many respects upon which the separation of this form could without any doubt be based.

Female (Fig. 3), 1·8 mm. long (on slide).

This form differs from *R. lobulata* as follows. Its head is much more rounded when viewed dorsally, and there are no small stout setae on the posterior margin of the vertex. The posterior margin of the ventral surface of the head bears only a few small setae, while those arising from the postgenae, in front of the anterior coxa, are much stronger than in *R. lobulata*. In other respects the dorsal and the ventral surfaces of its head do not differ from those of the species named.

The mesonotum of the new species is ovoid; the prescutum is sparsely covered with fine short setae; there is a bare space in the middle of the anterior part and in each postero-lateral corner of the prescutum; scutum with very

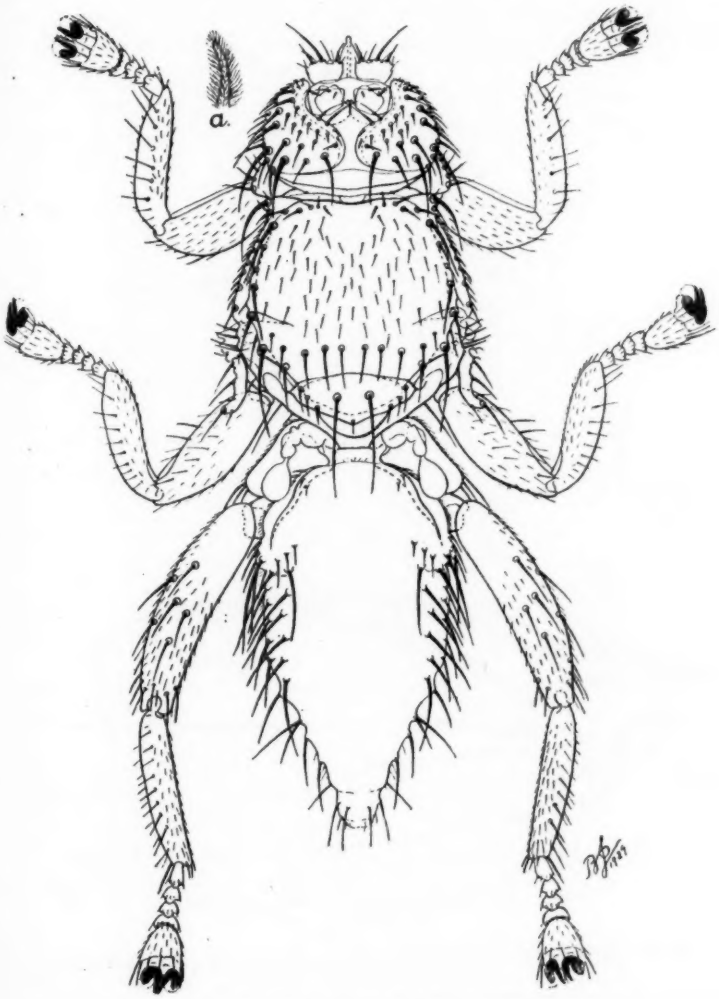


Fig. 3. *Raymondia planiceps* sp.n., female. *a*. arista of antenna.

few fine, short setae in the anterior part, and a more or less complete row of strong setae disposed across the middle. The posterior margin of the scutellum is angular, with one seta in the middle; the strongest setae arise from the middle of the scutellum; in each lateral part there are three short setae. Each

sternoplevra is twice as long as broad, sparsely covered with fine short setae (Fig. 4 A). Legs presenting no distinctive characters.

Wings (Fig. 4 B), 1·7 mm. long. Alula vestigial with single terminal seta; humeral vein with only one seta; no seta in the costal cell; few in the marginal

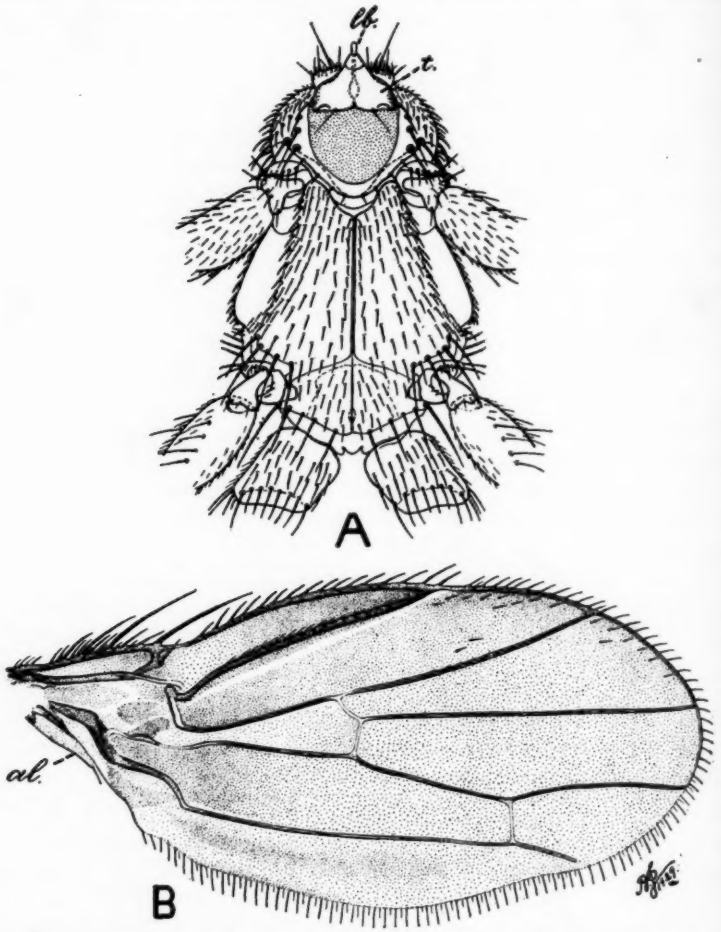


Fig. 4. *Raymondia planiceps* sp.n. A, ventral view of head and thorax; B, wing.

and the submarginal cells; fifth longitudinal vein bent at a right angle in its basal part.

Setae of the ventral surface of the abdomen stronger than in *R. lobulata* passing abruptly into very small ones in the antero-lateral parts; the most posterior part of the ventral surface, in front of the apical cone, is densely covered with short setae.

Raymondia pagodarum Speiser, 1900.

Raymondia huberi Schiner, 1868 (*nec* Frauenfeld, 1855).

This species may be very easily distinguished from all the others by the presence of a very clear, complete mesonotal suture, a peculiar shape of the calipteron (Fig. 5, *ca.*) and the curvature of the fourth longitudinal vein of the wings.

Previous record: according to Kessel (1925) "Off *Hipposideros speoris speoris* from Ceylon; off *H. larvatus* (or *Rhinolophus malayanus*) from the

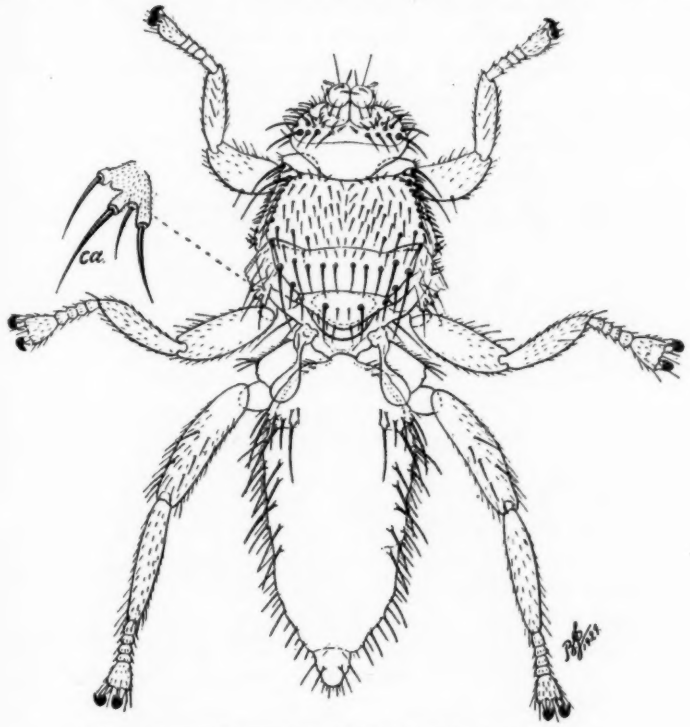


Fig. 5. *Raymondia pagodarum* Speiser, female. *ca.* calipteron.

Malay Peninsula; off *Emballonura nigrescens* from Molucca Islands and Amboina; off ? *Rhinolophus affinis* from Ceylon."

Present record: off undetermined bat from Chungking, China.

Female (Fig. 5), body 1·4 mm. long (on slide).

Head slightly quadrangular when viewed dorsally; subregions of vertex indistinct; one very strong seta in each postero-lateral part of the vertex; anterior to each of these there is one seta half as long; about seven small setae on each side of the anterior border and behind the antennae; six moderately

strong ones at the posterior border and two strong and several small ones on each lateral margin. Three strong and two much weaker setae on the inner border of each postgena, one in each lateral part, in front of the anterior coxa, and two much shorter ones in front of this; a few very small setae along the posterior margin (Fig. 6 A). The antero-lateral part of the postgena and the gena densely covered with very small setae. Palps small; theca of the labium as long as broad in its posterior part.

Thorax with very distinct, complete mesonotal suture; prescutum moderately covered with short setae, its lateral edges fringed with slightly stronger setae; posthumeral setae as long as the prescutum; humeral processes with one long and several very much shorter setae. Scutum with very few small setae in the anterior part and with a distinct row of much stronger setae arising

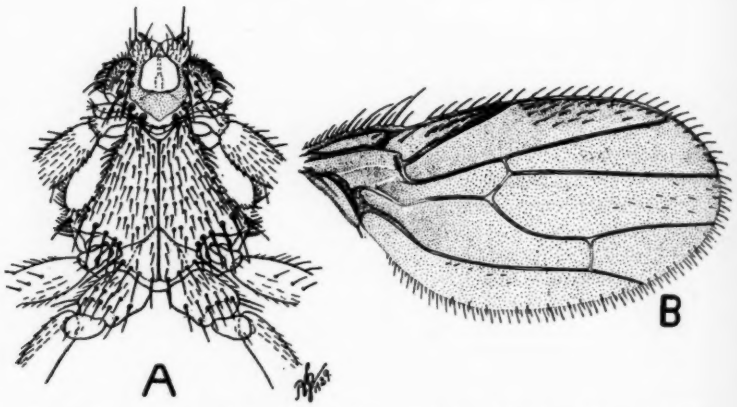


Fig. 6. *Raymondia pagodarum* Speiser. A, ventral view of head and thorax; B, wing.

nearer to the mesonotal suture than to the scutellum. Posterior border of the scutellum rather narrowly rounded, and bearing several short setae; strong scutellar setae far apart in the middle, two small ones between them and a slightly stronger one in each lateral corner. The sternoplevrae moderately covered with fine setae.

Legs presenting no distinctive characters.

Wings (Fig. 6 B), 1·3 mm. long; humeral vein with one seta; subcostal cell with a row of setae along the middle and a single one close to the humeral vein; marginal cell with many setae in its distal half. Fourth longitudinal vein strongly bent downwards after uniting with the anterior cross vein, forming an enlargement of the basal part of the first posterior cell; alula vestigial with single terminal seta.

Abdomen with a small apical cone, bearing two dorsal and two lateral moderately long, thin setae; ventral surface of the abdomen covered with short setae.

Male, 1·3 mm. long. Resembles female in all respects except in the apical cone of the abdomen, which is very large, sparsely covered with short setae ventrally, with two long and two slightly shorter setae in the posterior part. Claspers of the genitalia weakly chitinised, one of them straight, the other much broader and curved; apodeme of the penis very thin and longer than the other apodeme.

Raymondia quadriceps sp.n.

Three specimens were examined, the type, a female, and two paratypes, males, off undetermined bat from British Somaliland. They were preserved under the name of *R. lobulata* in the collection of the British Museum (Natural History Department). Their identification has undoubtedly been based upon the presence of a well-developed alula. Nevertheless, this species differs markedly from *R. lobulata* in its general appearance and the structure of the head, the labium and the thorax.

Female (Fig. 7), body 1·8 mm. long (on slide). Head distinctly quadrangular, narrower than the anterior part of the thorax; subregions of the vertex indistinct; eight very strong setae arise from the vertex: one in each postero-lateral part and three from each lateral border which also bears a very few small setae; two moderately strong setae in the middle of the posterior part and two practically of the same size behind each antenna, the rest of the surface of the vertex bears a few small setae; the posterior border fringed with short but stout setae. Ventral surface of the head with strong setae arising from the inner margin of each postgena and very small setae on the posterior margin; the anterior part of each postgena and the gena densely covered with fine short setae. Palps comparatively large; theca of the labium as long as broad.

Thorax rounded when viewed dorsally; the prescutum moderately covered with short setae; the mesonotal suture not quite distinct in the middle; humeral processes very small, each with one very strong and several small setae. Scutum with small setae in the anterior half, somewhat longer ones in the posterior and two very long ones in the middle; the posterior border of the scutellum broadly rounded and non-setose, two strong scutellar setae arise in the posterior half, there are three small setae above and three between them; external to these are two small setae and one stronger one in each lateral corner. Sternoplevrae densely covered with small setae.

Wings of the female (Fig. 8 B), 1·4 mm. long; broad; alula well developed with several setae. Humeral vein not quite distinct with single seta; subcostal cell with one seta close to the humeral vein and three in the middle; marginal and submarginal cells with few setae in the distal part.

The tergite-like portion of the abdomen has a slightly thickened and sinuated edge between its lateral lobes; the apical cone of the abdomen as broad as long, with four strong lateral setae; ventral surface of the abdomen covered with small setae, these become longer in the posterior and postero-lateral parts and very much smaller antero-laterally.

Male (Fig. 8 A), 1·4 mm. long, resembles the female in all respects, except in the apical cone of the abdomen, which is much larger, twice as broad as long and not quite distinctly separated from the rest of the abdomen on the ventral

Fig. 7. *Raymondia quadriceps* sp.n., female.

surface. It bears four very long and strong setae. The sternite-like portion forms a distinct, narrow plate which extends across the abdomen.

Genitalia with very short claspers; the apodeme of the penis about three times as long as the other apodeme and bent to the posterior part of the abdomen.

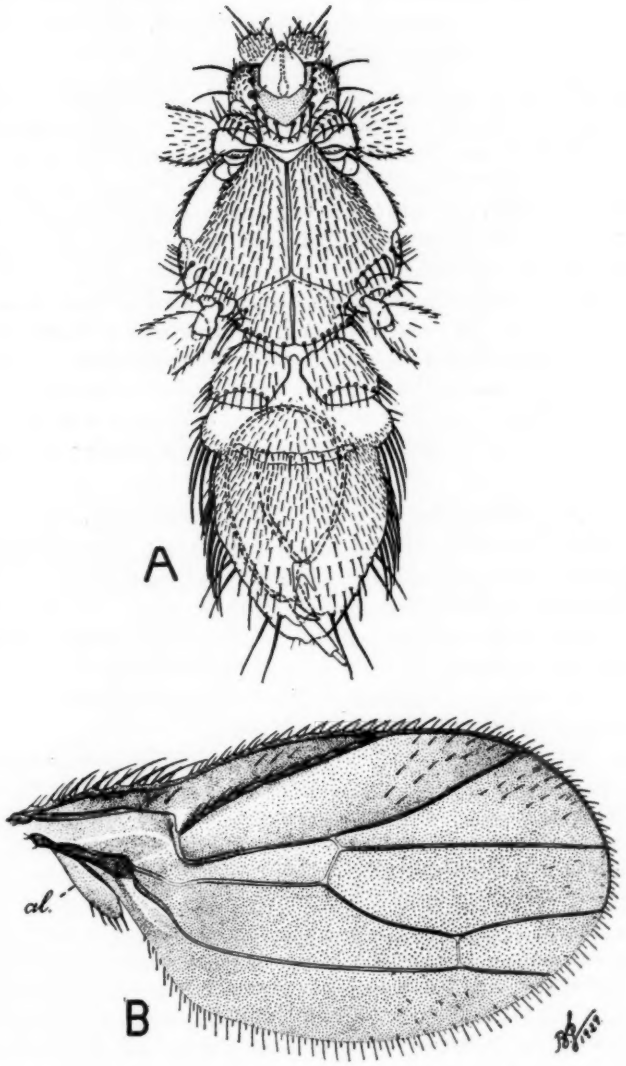


Fig. 8. *Raymondia quadriceps* sp.n. A, ventral view of body of male; B, wing.

Raymondia huberi Frauenfeld, 1855.

Strebla huberi Kolenati, 1856.

Strebla africana Kolenati, 1857.

On examining the specimens of *R. huberi* from the collection of the British Museum (Natural History) it was found that two forms were represented: in one the prescutum and the scutum are scantily covered with small setae; in the other these sclerites are distinctly more setose, while all the other setae of the body are longer. Speiser (1900), who examined Frauenfeld's specimens from the Vienna Museum, pointed out in his redescription of *R. huberi* that the prescutum is only slightly bristled on the surface. However, the female specimen received by me from the same institution had the prescutum densely covered with setae. As regards the original paper of Frauenfeld (1855) it is impossible to discover on what form his description was based. In view of this discrepancy I propose to refer the forms corresponding to Speiser's description to the typical form, and to separate as a new subspecies those in which the prescutum is very setose and all the other setae of the body are longer.

Raymondia huberi huberi Frauenfeld, 1855.

This species has been recorded from Egypt, Abyssinia, British East Africa and Zululand. According to Falcoz (1924), it has been found on the following bats: *Triaenops persicus* Dobson, *Hipposiderus tridens* Geoffer, *H. caffer* Sunder, *Megaderma cor* Peters, and *Coleura arfa* Peters. I have examined six specimens from Zululand and three from British East Africa.

The length of the body (on slide) varies in the females from 1·3 to 1·6 mm., in the males from 1·2 to 1·5 mm.

Head slightly quadrangular when viewed dorsally, narrower than the anterior part of the thorax, subregions of the vertex indistinct; the vertex bears one strong seta in each postero-lateral part, two somewhat weaker in the middle not far from the anterior margin and three on each lateral margin, which bears also a few small setae; behind the antenna there are one moderately strong and several small setae. On the ventral surface of the head each postgena bears several strong setae along the inner margin, one very strong one in the lateral part and anterior to this two much smaller ones; the antero-lateral part of the postgena and the gena with very small setae. Palps small; the theca of the labium a little longer than broad.

Thorax broader than long; anterior portion of the prescutum almost bare, the remaining portion scantily covered with small setae which become slightly longer in the posterior part; the mesonotal suture incomplete in the middle. Scutum with very few small setae and two much stronger ones in the middle. Scutellum rather broadly rounded, with a pair of strong setae arising from the middle, six very small ones on the posterior margin, of which two arise in the middle and two in each lateral part; a somewhat stronger seta arises from

each lateral corner. Sternoplevrae rather sparsely covered with fine setae. Humeral processes prominent, with one very long strong seta, another about half as long and a few very short.

Wings (Fig. 10), from 1·2 to 1·3 mm. long (on slide). Humeral vein with only one seta, no seta in the subcostal cell, very few in the marginal and the submarginal cells; fourth longitudinal vein nearly straight between the anterior and the posterior cross veins; the first posterior cell broad at the posterior

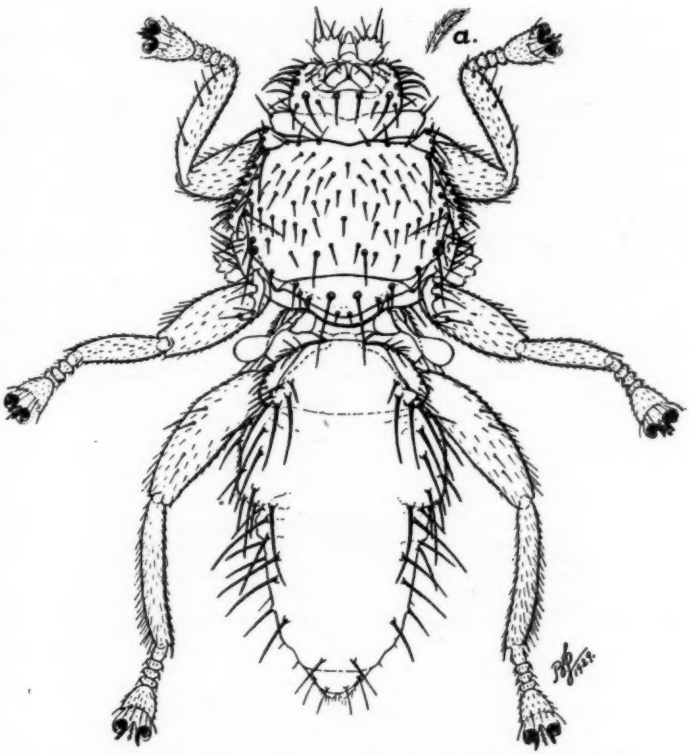


Fig. 9. *Raymondia huberi huberi* Frauenfeld, female. *a.* arista of antenna.

cross vein, the fifth longitudinal vein not reaching the wing margin. Alula vestigial with single terminal seta.

Abdomen with small apical cone which is nearly twice as broad as long at the base, bearing four moderately long thin setae; two strong setae on the dorsal surface of the abdomen in front of the apical cone; the tergite-like portion is separated from the rest of the dorsal surface by a very indistinct line; the setae of the ventral surface of the abdomen gradually pass into slightly smaller but not numerous ones in the antero-lateral parts; in some specimens the posterior third of the ventral surface is covered with very few setae.

Male (Fig. 10 C), length of the body from 1·2 to 1·5 mm. Differs from the female as follows: Setae of the ventral surface of the abdomen gradually become much smaller and more numerous on the lateral surfaces. Apical cone

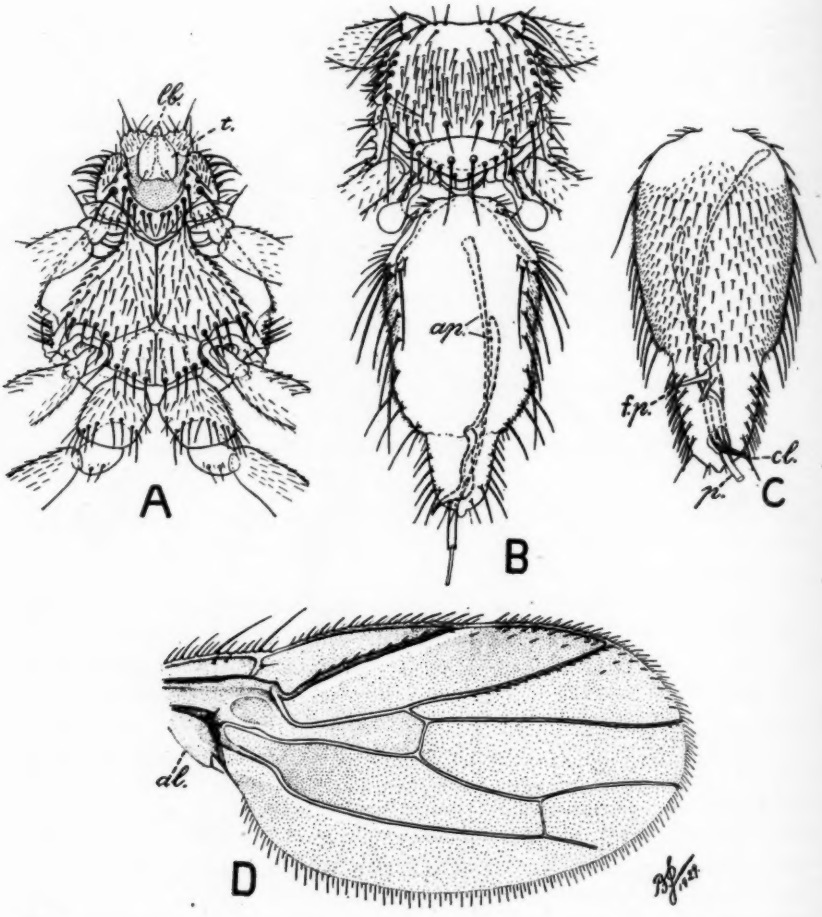


Fig. 10. *Raymondia huberi huberi* Frauenfeld. A, ventral view of head and thorax; C, abdomen of male; D, wing; B, dorsal view of thorax and abdomen of male of *Raymondia huberi setosa* subsp.n.

chitinised and about one-third of the total length of the abdomen, its dorsal and ventral surfaces bare, while laterally it carries many moderately strong setae. One of the claspers of the genitalia much longer and curved; apodeme of the penis twice as long as the other apodeme.

Raymondia huberi setosa subsp.n.

Two males off broad-eared bat, from Aden (Fig. 10 B), length of the body (on slide) 1·3 mm. This form differs from the male of *R. huberi huberi* in the following characters: all setae tend to be longer; the setae on the prescutum are more numerous; scutum with about six small setae in each lateral part, one of the same length in the middle, between two moderately strong ones; scutellum with the same number of setae as in the typical form, but the small setae stand at equal intervals from each other on the posterior margin of the scutellum. Each sternoplevra bears setae of same length as those of the prescutum.

Wings 1·2 mm. long; compared with the typical form they have more setae in the marginal cell, while the fifth longitudinal vein ends much farther from the margin of the wing. Furthermore, the apical cone of the abdomen has few setae on the ventral surface and the setae of the lateral parts are less numerous.

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ON THE SYNONYMY OF THE GENERA *TREMIORCHIS* MEHRA AND NEGI 1926 AND *CENTROVITUS* BHALERAO 1926, WITH THE DESCRIPTION OF *TREMIORCHIS VARANUM* N.SP.

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(With Plate XXXI and 10 Text-figures.)

Introduction.

Bhalerao (1926) described a trematode from the common Indian frog, *Rana tigrina*, which he placed in a new genus *Centrovitus* and a new species *pentadelphi*. Mehra and Negi (1926) gave a fuller account of the same form, from the same host and practically the same locality, under the designation *Tremiorchis ranarum* n.g., n.sp.

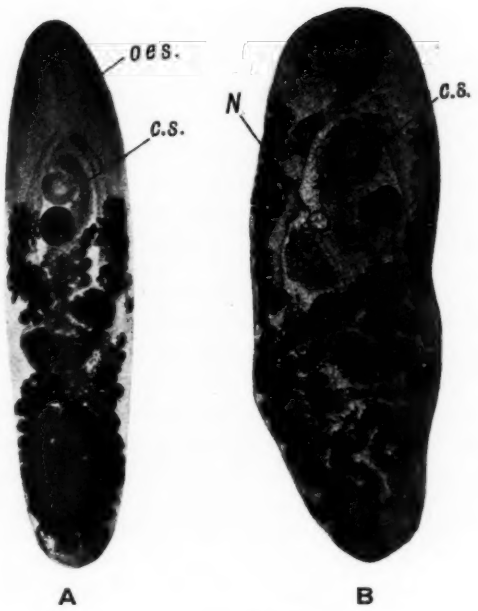
Although both the above communications appeared in print simultaneously in this *Journal* (**18**, 154–9, 168–81), the identity between the descriptions of *Centrovitus* and *Tremiorchis* appears to have escaped notice, at the time of publication. But it is strange that the authors concerned have not published any note, so far as I know, concerning this fact, which ought to have struck them long ago. They are therefore responsible for the insertion of both *Centrovitus* and *Tremiorchis* as distinct genera in some important publications[1].

Tremiorchis and *Centrovitus* are synonymous.

A study of the authors' descriptions above referred to and the text-figures given therein unmistakably indicates that both of them refer to the same form, in spite of the undernoted differences:

(i) Bhalerao locates the spines on the anterior two-thirds of the body. Mehra and Negi, however, give them a longer extent, stating that, in the undeveloped individuals, the spines densely cover the whole body but, in mature ones, only four-fifths of it; it is to be noted that their figures (p. 173, Text-figs. 1 and 2) do not support their own account but that of Bhalerao. With thirty mounted specimens before me, I find that the spines are disposed and shaped as described by Bhalerao (see my Fig. 5).

[1] *Zoological Record* (1926). Section VI. Vermes. **63**, 42 and 48.
Kükenthal, W. and Krumbuch, T. (1928). Vermes Amera. In *Handbuch der Zoologie* (Berlin), **2**, 112–13.



Photomicrograph of balsam mounts.

Fig. A. *Tremiorchis ranarum*. The vitelline groups run into one another. ×22.

Fig. B. *Tremiorchis varanum* n.sp., adult. ×45.

Key to lettering of Plate: *c.s.* cirrus sac; *oes.* oesophagus; *N.* a nematode inside the worm.

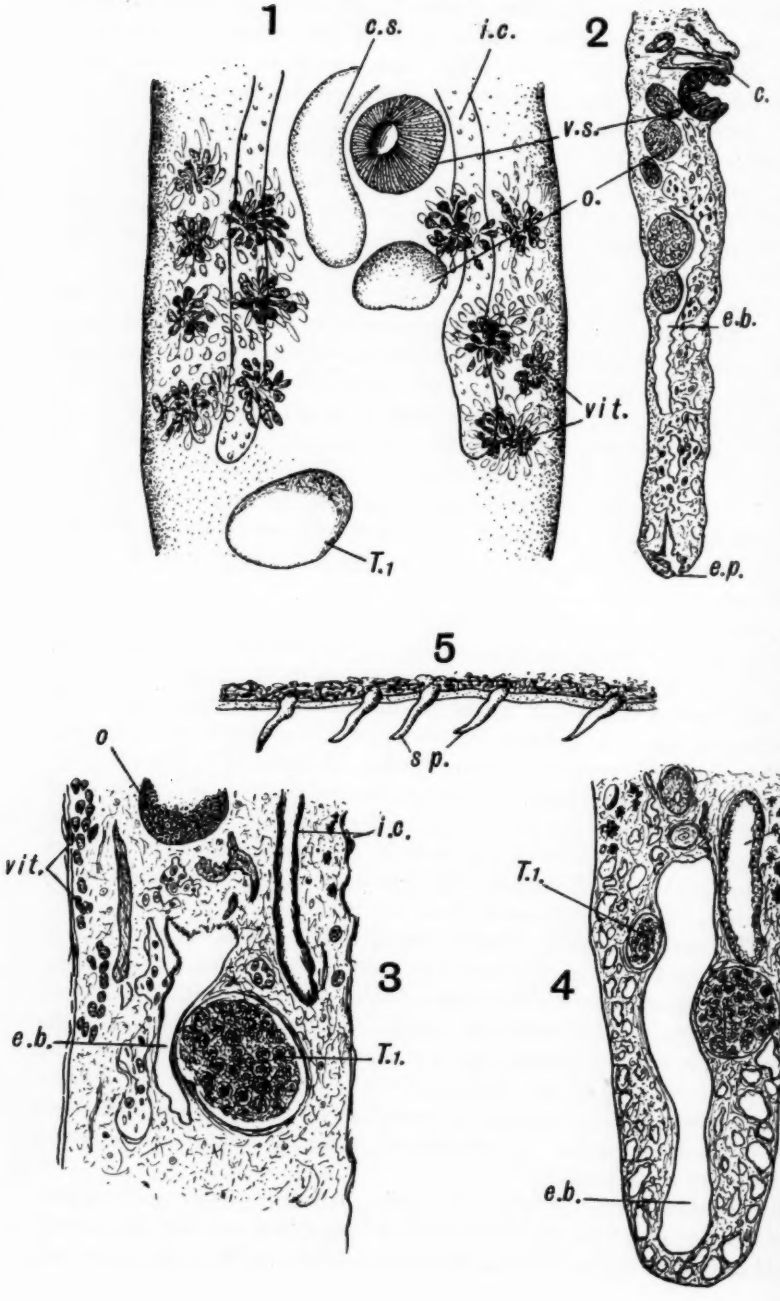
(ii) In the matter of the position of testes I again agree with Bhalerao that both of them lie in the posterior half of the body. The specimen sketched by Mehra and Negi (Text-fig. 2), on which they appear to have based their account, was probably more contracted in front and hence they say: "The left testis lies anteriorly about the middle of the body; it is situated more in the anterior half of the body than in the posterior half." In describing the excretory system, however (p. 177), they themselves have thrown correct light on this, as is evident from the sentence, "the bladder occupies nearly half the length of the body" and "extends forwards beyond the testes." Their Text-fig. 8 actually shows the anterior testis lying distinctly behind the middle of the body.

(iii) The cirrus sac in *Centrovitus* is said to lie on the right side of the ventral sucker, but in *Tremiorchis* it is described to be "on the left, seldom on the right side of the ventral sucker." This difference is immaterial, because the cirrus sac is a large structure which, in life, arches over the dorsal wall of the muscular sucker. In pressing the specimens it is but natural for it to get shifted a little to the right or left of the sucker according to the direction of the pressure exerted. In lightly pressed mounts and in vertical sections I have observed it to be nearly always median in position. These authors' figures, moreover, support this view in that they indicate the ventral sucker also displaced somewhat from its natural position, which is always median in the living worm.

(iv) The vitellaria, according to Bhalerao, form five groups on each side "from the posterior end of the ventral sucker to the end of the intestinal caeca, the number of acini in each group varying from 14 to 30." According to the joint authors also they occur in groups, but extend forwards, beyond the ventral sucker, up to the point of bifurcation of the intestinal caeca. Judging from their figure, the number of groups on each side seems to be 10 or 12, each consisting of a few acini only. In most of the preparations before me, they extend forwards only to the level of the hinder margin of the ventral sucker, rarely reaching its middle level, but never do they extend to the level of the bifurcation of the intestinal caeca. My figures (Pl. XXXI A and Text-fig. 1) show their extent and arrangement more correctly.

(v) The excretory system presents a marked difference in the two descriptions under consideration. *Centrovitus* is credited, by its author, with possession of a terminal excretory pore and a Y-shaped bladder with a long stem which bifurcates, a little behind the posterior testis, into two arms. But the authors of *Tremiorchis* mention that the excretory pore, in their genus, lies somewhat in advance of the hinder end of the body, and that the bladder "extends forward beyond the testes, reaching very nearly to the hinder end of the seminal receptacle....Its anterior end is slightly dilated and produced into two very small pockets."

Having made a careful study of the species, in the living state and from good horizontal sections, I can say with certainty that Mehra and Negi are correct. The bladder does extend beyond the anterior testis, before it bifurcates into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the two very short cornua (Text-figs. 2 and 3), which can hardly be detected when the animal is well expanded (Text-fig. 4). But the excretory pore is terminal and lies in a shallow depression facing ventrally (Text-fig. 2), or, at most, it may be said to be ventro-subterminal.

Therefore *Tremiorchis ranarum* and *Centrovitus pentadelphi* are synonymous names, and the first should stand, having priority, because Mehra and Negi (1925), before the final publication of their paper in *Parasitology* (1926), had read a paper on this form at the Indian Science Congress, Benares, and an abstract thereof appeared in the proceedings of that body for the year 1925. The correct name of the species is thus *Tremiorchis ranarum* Mehra and Negi 1925, and its definition is as follows:

Specific diagnosis of T. ranarum (emended): Body 1·7–5·6 mm. long, 0·3–1·32 mm. broad, anterior two-thirds covered with spines of average length 25μ. Oral sucker subterminal, smaller than ventral; ratio 3 : 4, ventral near anterior third of body. Prepharynx very short, pharynx globular, 0·12–0·19 mm. in diameter, oesophagus narrow, moderately long; intestinal caeca wider than oesophagus reaching as far as middle of body. Testes close together posterior to intestinal caeca, obliquely behind one another. Cirrus sac long, curved over ventral sucker, extending somewhat behind it. Vesicula seminalis narrow, elongated, in posterior third of cirrus sac. Ductus ejaculatorius short, muscular; pars prostatica distinct. Cirrus thick-walled, eversible, more than half as long as its sac. Genital pore close in front of ventral sucker. Ovary rounded, posterior to cirrus sac, average diameter 0·3 mm. Shell gland central, behind ovary. Receptaculum seminis prominent, kidney-shaped, nearly as long as the ovary but only half as wide, partly overlapping shell gland. Laurer's canal long, open, joins oviduct through seminal receptacle. Uterus in ascending and descending branches, much convoluted, filling space behind testes. Excretory pore terminal or slightly ventro-subterminal; bladder long, extending beyond anterior testis; horns very short or rudimentary. Eggs light brown, operculated 25–35μ by 12–18μ. Habitat: gut of *Rana tigrina*.

In view of the foregoing and in the light of the new species described hereafter, the generic diagnosis proposed by Bhalerao (1926, p. 158) and the diagnostic characters given by Mehra and Negi (1926, p. 179) in the table of genera of the sub-family Plagiorchinae also need modification. This follows after the anatomy of the new species is considered (see p. 310).

Text-figs. 1–5. *Tremiorchis ranarum.*

1. Part of a photomicrograph of a balsam mount; vitellaria in distinct groups. ×80.
2. Vertical longitudinal section showing extent of excretory bladder and its opening. ×32.
3. Horizontal section indicating short cornua of bladder. ×50.
4. Horizontal section of another specimen with bladder fully stretched and horns invisible. ×80.
5. Portion of body-wall with spines. ×400.

Key to lettering of Text-figs. 1–5: *c.* cirrus; *c.s.* cirrus sac; *e.b.* excretory bladder; *e.p.* excretory pore; *i.c.* intestinal caecum; *o.* ovary; *sp.* spines; T_1 anterior testis; T_2 posterior testis; *vit.* vitellaria; *v.s.* ventral sucker.

Tremiorchis varanum n.sp.

Host: *Varanus bengalensis* and *V. griseus.*

Habitat: Small intestine.

Locality: Allahabad.

Fifteen mature specimens of this trematode were obtained from a *Varanus bengalensis* dissected on 7. IX. 1928 in the laboratory. Later, a young *V. griseus* was examined, but half a dozen immature *T. varanum* were collected therefrom. This fluke, the second species of the genus *Tremiorchis*, differs chiefly from the type species in the nature and extent of its body spines, in the ratio and the relative size of the suckers, in the length of its vitellaria and intestinal caeca, in the much more anterior position of its anterior testis and in the size of eggs.

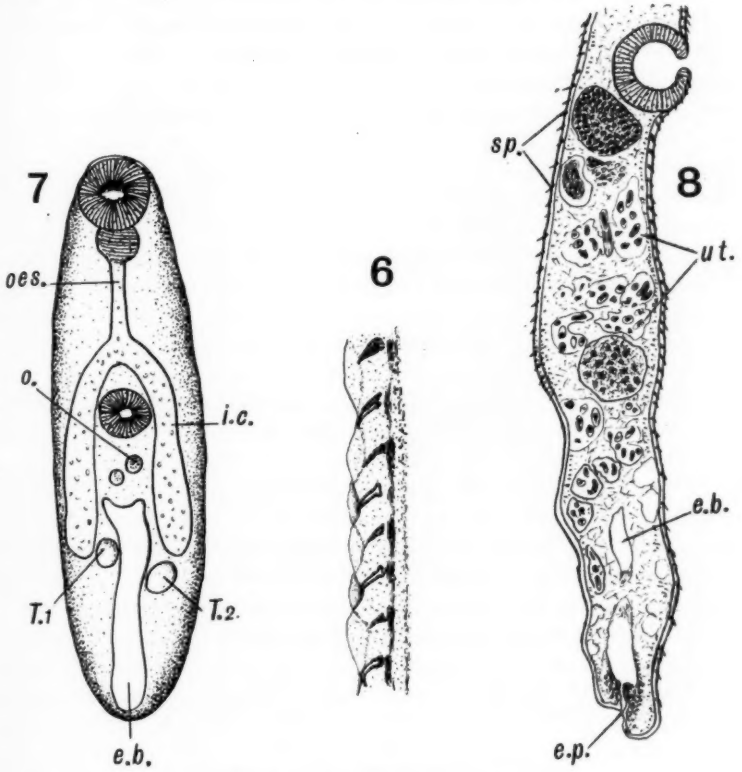
Description. The dimensions of the mature and immature specimens are given in Table I. The living worm illustrated in Pl. XXXI, fig. B, measured as follows:

		Breadth			
	Length	In front of ventral sucker	From ventral sucker to hind testis	Oral sucker (diameter)	Ventral sucker (diameter)
Expanded	1·68–2·1	0·24–0·28	0·42	0·21–0·25	0·18–0·21
Contracted	1·1	0·4	0·7	(Indistinct)	

The worm is white but the uterus, being full of light brown eggs, imparts a similar colour to the region of the body it occupies. The fully extended living distome is spindle-shaped, but, on fixation, the body ordinarily appears short to long oval with rounded extremities, depending upon the state of contraction.

The cuticle is covered with small but conspicuous spines which envelop the body up to the region of the second testis. Behind it they decrease rapidly in numbers but extend, on the ventral surface alone, at longer intervals to the posterior extremity (Text-fig. 8). The spines are distinctly smaller than in *T. ranarum* as shown in Text-figs. 5 and 6. The oral sucker is ventro-terminal, with thick muscular walls and, unlike that of *T. ranarum*, it is larger than the ventral sucker. The ventral sucker, in immature specimens, lies about midway along the body axis, but in adults it lies anteriorly one-third to one-fourth along the body-length according to the state of contraction when fixed. A very short prepharynx, easily perceptible in longitudinal sections and in life, interposes between the anterior sucker and the globular pharynx which is nearly one-third as large as the sucker. The oesophagus is fairly long. In young individuals it runs to about the end of the first third of the body, but in adults it extends from one-fourth to one-fifth the body length. The intestinal branches, as in the type species, terminate near the commencement of the last third of the body in immature forms, but in adults they end shortly behind the middle of the animal.

The genital pore lies just in front of the acetabulum in the median line or slightly inclined to one side. The testes lie obliquely one behind the other. When young, they are oval and close to the ends of the intestinal caeca as shown in Text-fig. 7; when adult their outline is not so regular and their relative position also somewhat different—the anterior testis is ovoidal and lies anterior



Text-figs. 6–8. *Tremiorchis varanum* n.sp.

6. Part of body-wall with spines. ×400.
7. An immature mounted specimen. ×100.
8. Vertical longitudinal section showing excretory pore and extent of spines. ×50.

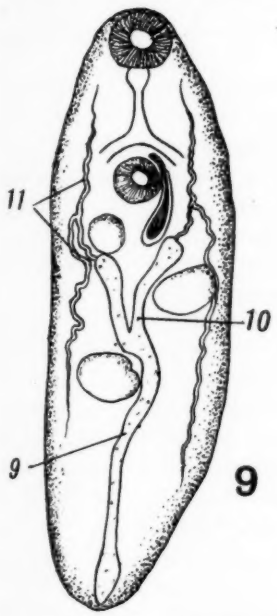
Key to lettering of Text-figs. 6–8: *oes.* oesophagus; *ut.* uterus; other letters as in Text-figs. 1–5.

to the middle of the body, the intestinal caeca extending a little behind its hinder margin. The posterior testis is more median than the anterior, a little larger, and transversely elongated; it always lies behind the middle of the body and the intestinal caeca terminate just in front of or near its cephalad border. In some preparations (Pl. XXXI, fig. B) it shows quite a regular outline, concave in front and convex behind. *T. varanum* differs markedly from *T. ranarum* in

the comparatively forward location of its anterior testis. The cirrus sac is a characteristically curved, elongated structure which is nearly twice as long as the oral sucker over which it bends forward along its dorsal aspect. On pressing the specimens, during fixation, the sac is displaced from its natural position more often to the right than to the left of the acetabulum. Within it is enclosed a long seminal vesicle, a short pars prostatica communicating with a protrusible cirrus through a narrow ejaculatory duct. The ovary is pear-shaped, close behind the ventral sucker and submedian in position. In pressed mounts it appears a little more sideways than natural, either on the same side of the sucker as the cirrus sac or on the opposite side. The oviduct arises from the inner, pointed surface of the ovary and is soon joined by a wider duct from the seminal receptacle and a narrower one from the median vitelline reservoir. It then turns backward and continues into the ootype which is surrounded by the inconspicuous diffuse shell glands. Laurer's canal (Text-fig. 10) is a long narrow tube which, starting from its aperture on the dorsal surface behind the vitelline reservoir, dips inward in a spiral manner and then takes a short transverse course. After one more descent, by a backwardly directed loop, it again runs a little transversely and dilates into the receptaculum seminalis. The latter is a long wavy sac, posterior to the ovary and is doubled upon itself before narrowing into the duct which opens into the oviduct. The uterus has three or four coils between the intertesticular space, but behind the posterior testis it is thrown into numerous coils completely filling the body up to the end. Finally it passes forwards between the testes and after one or two short convolutions behind the cirrus sac runs along its inner margin, then crosses it to the outside, and turns in front of it to open into the genital atrium. The vitellaria also differ from those of *T. ranarum* in being longer and not arranged into distinct groups. They form large follicles along the entire length of the intestinal branches which they overlap but partly. In some cases, anteriorly, they appear to extend a little ahead of the level of the intestinal bifurcation, whereas posteriorly, the left one stops short of the caecal end. The eggs are oval, of a yellowish brown colour and measure 0·025 by 0·014 mm. to 0·033 by 0·16 mm. in size.

The excretory bladder is very long and nearly straight. In young flukes (Text-fig. 7) it is about one-third as long as the worm and after crossing the anterior testis, bifurcates into two short horns. Owing to the more rapid growth of the hinder part of the body during maturity, its length in the adult is considerable. Passing ahead of both the testes it divides into two prominent cornua (Text-fig. 9), one running obliquely outwards behind the cirrus sac, and the other extending to the post-ovarian border. Each horn was observed in the living, undeveloped individuals, to receive a long anterior and a doubtful, short, posterior collecting duct. The former, a little behind the ventral sucker, is joined by another long tubule from the hinder outer part of the body. The bladder leads to the outside by a short narrow duct opening by a median dorso-subterminal pore (Text-fig. 8).

Specific diagnosis of T. varanum *n.sp.* Body 1·5–3·0 mm. long, 0·4–0·7 mm. broad. Oral sucker larger than ventral: latter at about $\frac{1}{4}$th the body length from the anterior end. Prepharynx very short, pharynx small globular, oesophagus long; intestinal branches reach a little behind mid-body. Testes oblique, one behind the other, separated by a moderate distance; anterior in first half, posterior in second half of body. Cirrus sac elongated, curved over acetabulum, twice as long as the latter. Vesicula seminalis elongated, swollen at base of cirrus sac; pars prostatica, ductus ejaculatorius short, cirrus long eversible.



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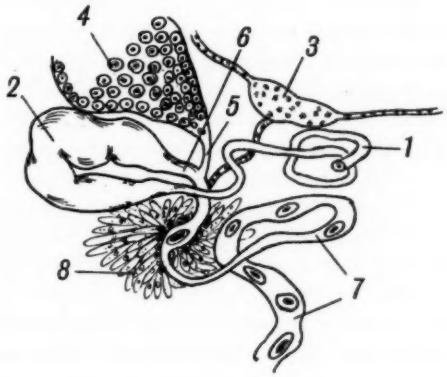

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Text-figs. 9 and 10. *Tremiorchis varanum* n.sp.

9. Diagram of excretory system as seen in living specimens.

10. Diagrammatic representation of female genitalia and related ducts.

Key to lettering of Text-figs. 9 and 10: 1, Laurer's canal; 2, receptaculum seminalis; 3, vitelline reservoir; 4, ovary; 5, oviduct; 6, duct of seminal receptacle; 7, uterus; 8, shell gland; 9, excretory bladder; 10, cornua of bladder; 11, excretory ducts.

Ovary pear-shaped, nearly median, close behind ventral sucker. Shell gland central, diffuse, posterior to ovary. Receptaculum seminalis long, twisted, alongside shell gland. Laurer's canal long, open, dilates into the seminal receptacle. Vitellaria, from level of bifurcation to end of intestinal caeca, not arranged in groups. Uterus convoluted filling space behind testes to end of body. Genital pore just in front of ventral sucker. Eggs yellowish brown, 0·025 by 0·014 mm. to 0·033 by 0·016 mm. Habitat: intestine of *Varanus bengalensis* and *V. griseus*.

GENERIC DIAGNOSIS AND SYSTEMATIC POSITION OF *TREMIORCHIS*.

It is evident from what has been said above, that the diagnosis of the genus given by the original authors does not hold good, in all respects. Moreover, *T. varanum*, n.sp., herein described for the first time, differs from the type-species (*T. ranarum*) in certain important anatomical features which have to be considered in the definition of the genus. For instance:

(i) *T. ranarum* bears spines only on the anterior two-thirds of the body but *T. varanum* has them on the whole body, at least on the ventral surface.

(ii) The oral sucker is smaller than the ventral in *T. ranarum*, but the reverse in *T. varanum*.

(iii) In *T. ranarum* both testes lie in the second half of the body, at the ends of the intestinal caeca, but in *T. varanum* the anterior testis is distinctly located in the first half, and anteriorly to the termination of the caeca.

(iv) The vitellaria in *T. ranarum* do not extend forward beyond the posterior border of the ventral sucker, but in *T. varanum* they reach the level of bifurcation of the oesophagus into the intestinal branches.

(v) The horns of the excretory bladder are very short and indistinct in *T. ranarum*, but they are fairly long and prominent in *T. varanum*.

I therefore propose the following altered definition for the genus.

Generic diagnosis of Tremiorchis (emended). Body spiny, of moderate size, elliptical or ovoidal, extremities rounded. Oral sucker subterminal, larger or smaller than ventral; prepharynx very short, pharynx globular; oesophagus moderately long; intestinal caeca terminate about middle of body. Testes obliquely behind one another, anterior in first or second half of body, posterior always in second half. Cirrus sac curved like a half moon over ventral sucker. Ovary median or submedian, behind ventral sucker. Vitellaria lateral, along part of or whole length of the intestinal caeca. Receptaculum seminalis large, with long Laurer's canal arising from it. Uterus voluminous, coiled as far as posterior end. Excretory bladder with a very long stem extending cephalad to both testes, horns short or moderately long; pore terminal or subterminal, dorsal or ventral. Eggs nearly twice as long as broad, operculated.

Systematic position of Tremiorchis. The genus has already been assigned a place in the sub-family Plagiorchinae Pratt 1902 (= Lepodermatinae Looss 1899) of the family Plagiorchidae Lühe, syn. Lepodermatidae Odhner. This family, as very aptly remarked by Travassos (1929, p. 353), although lately much studied, needs careful revision, as there is no unanimity, among the various authors, concerning the position of some of its genera. In fact, what Nicoll (1914, p. 344) wrote about it fifteen years ago: "Practically no other family of Distomate Trematodes shows such a conflicting diversity of form and structure," still holds good. Baer (1924) attempted a classification of the family which has not been referred to by the authors of *Tremiorchis* or by the author of *Centrovitus*. Bhalerao compared his genus with *Plagiorchis* Lühe 1899, *Opisthoglyphe* Looss 1899, *Dolichosaccus* Johnston 1912 and *Brachy-*

saccus Johnston 1912, and distinguished it from them. Mehra and Negi instituted comparisons with *Lepoderma* Looss 1899, syn. *Plagiorchis* Lühe 1899, *Haplometra* Looss 1899, *Haplometroides* Odhner 1911 and *Astiotrema* Looss 1900. The last named genus had been separated by Baer (1924) into the sub-family Astiotrematinae. They also gave a clear analysis of the likenesses and differences between their genus and the two known genera of the sub-family Brachycoeliinae Odhner. But the distinctive importance in *Tremiorchis* of "ventral sucker somewhat larger than the oral sucker" now disappears, as in my new species (*T. varanum*) here described the reverse is the case. Poche (1925) does not wholly accept the classification of Baer, but with this we are not concerned at present.

Travassos (1929) has thrown some light on the relationship of the genera of this family as a whole, including the genus *Tremiorchis*. It is therefore necessary to look into his scheme as he has tried therein to reconcile the suggestions of Baer and Poche with his own views upon the phylogeny of the group. Of the six sub-families into which Plagiorchidae has been split up by him (including Baer's Reniferidae as a sub-family), the one with which we have to do, namely Plagiorchinae, is sub-divided as follows into four sections:

(*a*) *Plagiorchis* Lühe 1899; *Haplometra* Looss 1899; *Stiphlodora* Looss 1899; *Glossidium* Looss 1899; *Glossidiella* Trav. 1927; *Haplometroides* Odhner 1911; *Opisthoglyphe* Looss 1899; *Aptorchis* Nicoll 1914; *Pneumatophylus* Odhner 1911.

(*b*) *Styphlotrema* Odhner 1911; *Pachypsolus* Looss 1901.

(*c*) *Astiotrema* Looss 1900; *Glyphthelmins* Stafford 1905; *Tremiorchis* Mehra and Negi 1926; *Rudolphiella* Trav. 1925; *Dolichosaccus* Johnston 1912; *Brachysaccus* Johnston 1912; *Oistosomum* Odhner 1902; *Xenopharynx* Nicoll 1912; *Mediorhyma* Nicoll 1914; *Dasymetra* Nicoll 1911.

(*d*) *Enodiotrema* Looss 1900.

It is unfortunate that Travassos's paper does not enlighten us about the definition of his sub-families, neither has he given therein any clue as to the characteristics on the basis of which the subdivisions have been proposed. So far as *Tremiorchis* is concerned—leaving aside other features—it differs markedly, in the restricted length of its lateral vitellaria and shortness of the intestinal diverticula, from practically all the genera[1] of Plagiorchinae mentioned above, except *Mediorhyma* or *Mediorima* and *Stiphlodora* or *Styphlodora*. In the former the vitellaria are more or less like those of *Tremiorchis* and so are the testes and ovary, but the intestinal caeca are still shorter, the vesicula seminalis convoluted instead of straight and the genital pore more ahead. The latter, though much like *Tremiorchis* in the extent and location of the vitellaria, in the general arrangement of the genitalia and the disposition of the uterus, is readily distinguishable from it by its longer caeca and the situation of both testes comparatively more anteriorly. Another genus of the sub-family

[1] The paper dealing with Travassos's genus *Rudolphiella* 1925, is not available to me: hence I am unable to refer to it.

with short intestinal caeca is *Aptorchis*, but its vitellaria are very different from those of *Tremiorchis* and its testes wellnigh symmetrically placed. It is therefore clear that *Tremiorchis* is a very interesting genus in that it combines in itself features common to several genera of Plagiorchinae and probably also of some of Brachycoeliinae, and it may throw more light on their relationships. As it approaches nearest to the genus *Mediorhyma* of group (*c*) and *Stiphlodora* of group (*b*), I am inclined to regard, in the absence of known reasons, the subdivisions proposed by Travassos as also unsatisfactory, if not unnatural. It will be very useful if some one were to undertake a comprehensive study of this family and unravel the knotty points once for all.

Table I.

Dimensions of mature and immature mounted specimens of T. varanum (mm.).

Mature	Length	Breadth	Distance of caecal bifurcation from anterior end	Caecal length	Oral sucker, diameter	V.S.* diameter	Distance of V.S.* from anterior end	Ovary, widest diameter	Distance of first testis from anterior end, and size	Distance of second testis from anterior end, and size
1	2·80	0·76 (near V.S.*)	0·55	1·14	0·26	0·21	0·80	0·17	1·4 (crumpled)	1·7 (0·18 × 0·2
2	2·10	0·60 (V.S.* to second testis)	0·31	0·84	0·19	0·17	0·46	0·15	0·84 (0·19 × 0·19)	1·15 (0·19 × 0·15
3	2·08	0·67 (near V.S.*) 0·7 (near testis)	0·34	0·88	0·25	0·21	0·44	0·16	0·88 (0·25 × 0·17)	1·28 (0·33 × 0·17
Immature										
4	0·93	0·25 (in middle)	0·32	0·36	0·12	0·09	0·44	0·025	0·67 (0·042 × 0·034)	0·71 (0·059 × 0·0
5	0·80	0·26	0·31	0·34	0·12	0·10	0·41	0·026	0·64 (0·042 × 0·031)	0·70 (0·057 × 0·0

* V.S. = ventral sucker.

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LIPONYSSUS SYLVIARUM INJURING POULTRY IN THIS COUNTRY.

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This mite was reported by Hirst in 1923 to be causing damage to poultry in Bedfordshire. He states that, although it had previously been found in pigeons, it had not before been recorded as a pest of fowls in this country. In the United States of America it has frequently occurred, and is regarded as a dangerous parasite, but Hirst's record seems to be the only one of its occurrence as a pest in the British Isles.

In October last a few fowl's feathers were received from a poultry farm at Dorchester containing a large number of mites in various stages of development, bearing a very close resemblance to *Dermanyssus gallinae*, but which, on more minute inspection, proved to be *Liponyssus sylviarum*. Unlike the red mite this species does not leave the host during the day but is to be found on the birds at any time, and more particularly on the abdominal parts, to which its attack is principally confined.

The Dorchester poultry farmer informs me that no hiding mites could be found on making a careful inspection of the poultry houses, nor were any to be found in the nest boxes or on the eggs. In this particular, the observation is at variance with that of Cutright (1929), who records an outbreak in New York, where the presence of large numbers of mites on the hen's eggs in the nest was the first indication of infection.

On the Dorchester farm the first appearance of disease was a general weakness shown by some of the birds, which increased to such an extent that the worst affected became unable to stand. It was in making an examination of these birds that the mite was first observed. By way of treatment Cutright reported very successful results from painting the perches with nicotine sulphate after attempted control by other methods had failed. In the outbreak here recorded, dressings of nicotine sulphate and paraffin were applied to the perches and similarly good results obtained. The mites disappeared after a second treatment and all fowls recovered without any deaths having occurred.

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(*MS. received for publication*, 19. xi. 1929.—Ed.)

STUDIES ON THE MORPHOLOGY AND BEHAVIOUR OF *BUXTONELLA SULCATA* FROM CATTLE AND OF *BALANTIDIUM COLI* FROM THE PIG.

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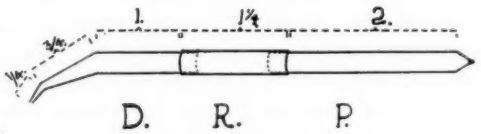
(With Plate XXXII and 6 Text-figures.)

During the fall and winter of 1928–9 the writer found an infestation in the caecum of 8 out of 32 head of cattle slaughtered at Jeanerette, Louisiana, with a ciliate that appeared to belong to the genus *Balantidium*. Since Protozoa belonging to this genus have been described from cattle in India by Cooper and Gulati (1926) and cysts reported from cattle in Europe by Sheather (1923), it appeared that the present writer's observation served to confirm the above discoveries and to indicate *Balantidium* infection of cattle in the United States. It was noted, however, that the ciliate of Louisiana cattle is larger than the balantidia reported by Cooper and Gulati. Twenty-five measurements by these investigators gave lengths of 60–120 microns and breadths of 44–90 microns. The length of 50 individuals measured by the writer was 95–238 microns and the breadth 79–180 microns. Other differences were a groove extending longitudinally and somewhat spirally from the cytostome throughout the length of the organism, and a second orifice near the mouth. It was also found that the ciliate travels with its mouth to the rear. The trophozoites did not occur in the faeces of normal living cattle, but were found in droppings of a cow that had a mild diarrhoea. This led to the present method of obtaining them by the administration of Epsom salts. When the writer noted the publication by Jameson (1926) on a ciliate from the caecum of cattle in England, it soon became apparent that the organism discovered at Jeanerette should be included in the genus and species, *Buxtonella sulcata*, which he proposed.

Material and methods of procedure.

The infections were all very light. They were noted when small amounts of faecal material in Syracuse watch glasses were diluted with normal saline and examined under the binocular microscope. The writer has found this method of faecal diagnosis superior to the common one of examining smears microscopically. Material can be looked over more rapidly, small clumps may be kept under observation while being torn apart with a needle, and organisms

may be found that would otherwise escape notice. Individuals may be picked out free from debris with a pipette and studied under higher magnification. The pipette shown in Fig. 1 has given excellent results. It consists of two small pieces of glass tubing connected by a small piece of rubber tubing (*R*), the proximal glass tube (*P*) is sealed at its free end and the distal tube (*D*) is drawn out at one end to a capillary and bent as illustrated. The device is held firmly as one holds a pencil, the meniscus being delicately controlled by pressure on the rubber tube with the thumb and index finger.



Text-fig. 1. Microisolation pipette, one-half actual size. *P.* proximal glass tube; *R.* rubber tube; *D.* distal glass tube. The dimensions given are in inches.

Microscopic technique.

While much information was gained from the study of living organisms and cleared unstained whole mounts, the cytological details were clearly observed in whole mounts and sections stained with iron haematoxylin. For the preparation of specimens the well-known centrifuge method was employed. The material was run from the fixative into 2 per cent. iron alum at 40° C. for 15 to 20 minutes and stained at the same temperature from 20 minutes to an hour or longer. Then, after washing five or six times in water, it was run into 1½ to 2 per cent. iron alum and allowed to remain for 36–42 hours. Shorter decolorising periods left the organisms black and hence opaque. When decolorised they were washed five or six times in water and allowed to remain overnight, after which the water was changed several times. Cedar oil was used for the final clearing of the dehydrated organisms. Whole mounts were studied between cover glasses by a method similar to that of Sharp (1914).

Sectioning of unicellular organisms is frequently accomplished by the gelatine capsule method of Metcalf (1909). The sparseness of ciliates in my material rendered this method unsuitable. The knife was injured by the grit and other debris of the faeces. The following original method was therefore adopted: A very small quantity of paraffin was melted in the depression of a hollow-ground micro-culture slide. A ciliate in cedar oil was then placed on the paraffin with the pipette and after inspection under the 4 mm. objective the slide was inserted into the paraffin oven. In about 30 minutes it was removed and the organism was located under the binocular microscope and kept in focus while the paraffin cooled. The ciliate was then removed from the slide in a block of paraffin cut by a sharp scalpel. The slide was cleaned, the depression refilled with paraffin (m.p. 58°–60° C.) coloured with Sudan III. The small block of white paraffin containing the organism was placed in the red paraffin

and the slide replaced in the oven. When it was removed the ciliate was again located and the paraffin allowed to harden as before. The organism was then cut out in a small rectangular block, and suitably orientated for cross or longitudinal sections. A Syracuse watch glass was then filled with melted paraffin (M.P. 48°–50° C.). Common parawax was used because available and having excellent ribboning qualities. When it had formed a hardened surface film, five or six of the small rectangular red blocks were orientated on its surface and covered 2 or 3 mm. deep by the use of cold and hot needles. It was then hardened immediately. An entire ribbon, which was usually cut at 3 microns, could easily be mounted on one slide, but it was found necessary to work in a cool place. The management of the Gulf Public Service Co. kindly permitted the writer to use for this work their ice storage room which is maintained slightly under 0° C. The sections of the ciliate were located under the binocular as soon as the strips became flattened on the slide and special attention was given to that part of the ribbon only. Some difficulty was experienced because of loosening of the smallest sections during deparaffining and staining. The method outlined by Gage (1927) of cementing them with collodion was effective. The collodion also prevented accidental crushing and loosening of sections during subsequent manipulations of the slide under the oil immersion. Much time and uncertainty was saved in the study of series sectioned by this method because of the following advantages over the gelatine capsule method: (1) the orientation of each organism when sectioned was definitely known; (2) there was no danger of confusing sections of two or more different organisms; (3) poorly fixed and otherwise distorted forms were never sectioned; (4) organisms could first be studied as whole mounts and then sectioned. The degree of flattening that was demonstrated in mounts that had been manipulated under oil immersion was surprising. In addition to *Buxtonella sulcata*, balantidia from the pig were sectioned. The advantages of definite information about the orientation of a given organism in the paraffin and about the relative position of any particular section along a given body axis will be best appreciated by those who have sought to make clear the confusing picture presented by sections through masses of organisms.

Review of the literature.

Jameson (1926) reported that the caecum in five out of eight cattle was heavily infested with the ciliate which he named *Buxtonella sulcata*; that the length of the organisms varied from 54 to 124 microns and the breadth from 40 to 72 microns and that the build of the body was stiff and stocky. He also noted and described a groove extending spirally throughout the length of the body and making a horseshoe-shaped curve around the oral end to lead to the mouth. He observed that the mouth is at the posterior end during progression and described an indentation near the mouth which he claimed does not open into the endoplasm. He stated further that the ciliate has two or more contractile vacuoles and described the endoplasm as heavily granular. The macro-

nucleus was bean-shaped and the micronucleus spheroidal, though certain changes of the former organelle suggested a nuclear cycle. Dividing forms were also studied and a brief description given of this process. The conclusion was reached that because of the thick cuticle, the general form of the body, and the form and position of the mouth the ciliate should be classified in the family Isotrichidae Bütschli.

Becker and Hsiung (1929) found cysts and trophozoites in the faeces of calves at the Iowa State Experiment Station which they classified as *Buxtonella sulcata*. The cysts were described, measured, and figured. They noted that the indentation near the mouth is visible through the cyst walls and that the measurements were within the size range reported by Jameson.

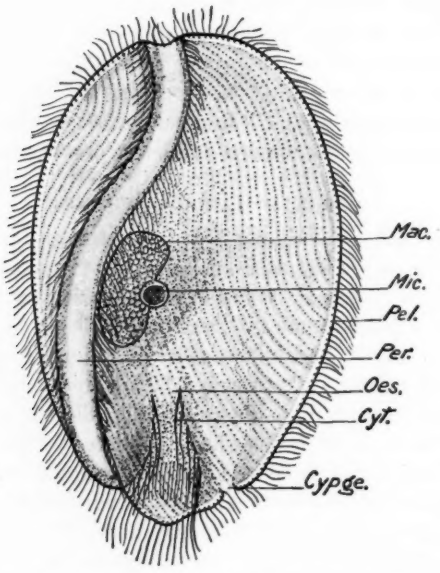
The descriptions of the trophozoites and cysts of *Buxtonella sulcata* given by the authors above cited enables others to identify this ciliate, but the present writer, through the technique he has employed, has arrived at an interpretation, differing from that of Jameson, through the study of sections, stained and unstained whole mounts and living organisms.

Size, shape, and relationship.

As previously noted the ciliates found in cattle at Jeanerette were larger than those of Jameson. The latter were of about the size of *Balantidium coli*, whereas it is shown in Text-figs. 3 and 4 that the mean breadth of 50 individuals was 116·6 ± 2·25 microns and the mean length 162 ± 3·2 microns, *i.e.* that the Jeanerette organisms were almost twice as broad and twice as long as were those found in England. Comparisons which were made by the writer with the balantidia found in pigs also illustrated these differences in size. However, Jameson states that the infections which he studied were heavy. On the other hand, those occurring at Jeanerette were all very light—about 10 c.c. of faecal material was carefully inspected to find the 50 individuals that were measured. This may account for the differences in size, the light infections resulting from less frequent division of individuals. As interpreted by the writer, the general morphology of *Buxtonella sulcata* is shown in Text-fig. 2. The figure is orientated as the organism is seen during locomotion, with the mouth to the rear, and a second orifice, which is believed to function as the cytopyge, occupies the position of the indentation described by Jameson. When viewed from the surface this orifice is an elongated slit but when seen in deep focus and in sections it appears as in Text-fig. 2 and in Pl. XXXII, figs. 2–5. It will be noted that the adoral, the oral, and the oesophageal cilia are specialised, being longer and heavier than the other cilia. Therefore the writer regards the groove as an elongated peristome and believes that the organism should be removed from the subclass Aspirigera and the family Isotrichidae, where it was placed by Jameson (1926), and included in the subclass Spirigera and the order Heterotrichida Stein (1859). This ciliate is, therefore, believed by the writer to be related to *Nyctotherus* Leidy (1849) and to *Balantidium* Claparède and Lochman (1858).

General anatomy.

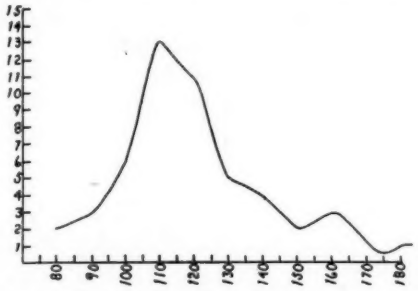
As indicated by the classification, which is proposed by the writer, and illustrated in Text-fig. 2 and in Pl. XXXII, figs. 1–12, the entire surface of the body of *Buxtonella sulcata*, except the floor of the peristome, is covered with fine cilia. The adoral cilia occur in parallel rows along the margins of the peristome. The lips of the cytostome are provided with oral cilia. The body cilia are arranged over the remaining surfaces of the organism in parallel rows. At the anterior end of the peristome the adoral cilia are only slightly longer than the body cilia, but caudad of this they become progressively longer, and near the



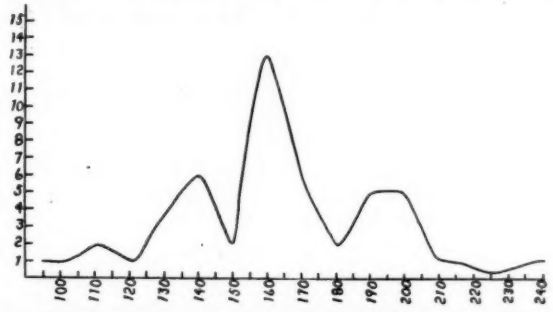
Text-fig. 2. Camera lucida drawing of *Buxtonella sulcata*, ×450. *Mac.* macronucleus; *Mic.* micronucleus; *Pel.* pellicle; *Per.* peristome; *Oes.* oesophagus; *Cyt.* cytostome; *Cypge.* cytopyge.

cytostome they are about twice as long. The oral cilia are slightly longer than the longest adoral cilia, there being no sharp boundary line between them. In this paper, for purposes of description, the grooved surface of the body which Jameson termed the dorsal will be called the ventral, other terms of orientation being the same as for bilaterally symmetrical organisms. Over the dorsal surface of the body the direction of the ciliary rows is longitudinal. On the ventral surface the peristome divides the rows in a peculiar manner, those on the right being parallel to the peristome, while those on the left meet it at acute angles. There is one row of body cilia that is longer than the others. It lies to the left of the peristome and stands out quite prominently in sections; in Pl. XXXII, figs. 1–7, it is seen to form a semicircle on the dorsal side of the posterior end of the

body around the peristome. The significance and function of this ciliary row could not be ascertained. As in other ciliates of the order Heterotrichida, the body of *Buxtonella sulcata* is enveloped by a thin transparent pellicle which is perforated by the cilia. Jameson refers to a thick cuticle. In descriptions of ciliates this term cuticle is usually a synonym for the pellicle. From the writer's point of view, however, the description by McDonald (1922) of the thin pellicle of *Balantidium coli*, as well as the ectoplasm of the latter ciliate, may be applied also to *Buxtonella sulcata*. As in *Balantidium coli*, the macronucleus



Text-fig. 3. Variation curve of the breadth of 50 individuals of *Buxtonella sulcata*. The lengths are noted on the abcissae and the number of individuals in each group along the ordinates.

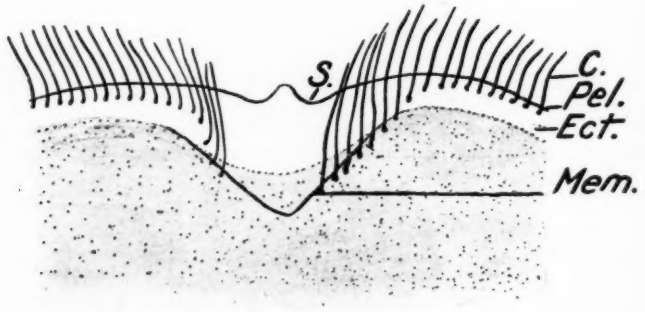


Text-fig. 4. Variation curve of the length of 50 individuals of *Buxtonella sulcata*, drawn to the same scale as Text-fig. 6.

and the micronucleus have no definite position within the cell. As indicated in Text-fig. 2 and in Jameson's figures, they resemble the homologous organelles of *Nyctotherus cordiformis*, the macronucleus being relatively shorter and plumper and the micronucleus relatively larger than in *Balantidium coli*. In stained specimens the nuclear membrane of the micronucleus is separated by a clear peripheral vesicle from the solid body of chromatin. Contractile vacuoles, though described and figured by Jameson, have not been noted by the writer in this ciliate of cattle, either in whole mounts or sections. On the other hand, the two vacuoles described by McDonald in *Balantidium coli* were found by the writer in every individual that was sectioned.

MINUTE ANATOMY.

It will not be necessary to describe the cilia, the pellicle, the ectoplasm, and the endoplasm of *Buxtonella sulcata*, because even though these structures were not described in detail by Jameson they do not appear to differ from homologous structures of other heterotrichous ciliates. Detailed descriptions of the peristome, cytostome, cytopharynx, cytopyge and neuromotor fibres are as follows: A cross-section through the peristome is shown in Text-fig. 5 (see also Pl. XXXII, figs. 1–7). It will be noted that within the groove the ectoplasm is considerably thicker than elsewhere, the pellicle being only slightly infolded, and that a secondary ridge, formed by an outfolding of the pellicle, lies within the groove about midway between the margins. A V-shaped membrane with its apex beneath this ridge appears to form an inner boundary layer of the ectoplasm. The origin and relationships of this layer were not determined be-



Text-fig. 5. Camera lucida drawing of a cross-section of the peristome of *Buxtonella sulcata* through about the middle of the body, ×1750. *Pel.* pellicle; *C.* cilia; *S.* secondary grooves of the pellicle; *Ect.* ectoplasm; *Mem.* membrane.

cause elsewhere over the body there is no definite line of demarcation between the ectoplasm and the endoplasm. That part of the pellicle that lies within the peristome is not perforated by the adoral cilia of which the ciliary rootlets appear to lie beneath the above-mentioned V-shaped membrane. These cilia pass through a zone of ectoplasm of considerable thickness to emerge through the pellicle on the lips of the peristome. Beginning at about the J-shaped bend, the adoral cilia extend entirely across the floor of the peristome (see Pl. XXXII, figs. 2–5). From this point to the cytostome they become increasingly longer, and considerably coarser than the other cilia. However, they do not appear to be composed of fused members as in *Nyctotherus*. The cytostome is a ciliated aperture, the walls of which are composed of the pellicle and the ectoplasm. In whole mounts (see Text-fig. 2) the oesophagus appears to extend to the region near the middle of the body, but if this organelle is ciliated at all the cilia occur only at the extreme oral end (see Pl. XXXII, figs. 1–7). These figures

are from a series of 38 cross-sections, hence of an organism that was about 120 microns long. The oesophagus is seen to be a vacuole within the endoplasm. The cytopyge is shown in Text-fig. 2 and in Pl. XXXII, figs. 3–6. It is an orifice situated in a deep groove, to the left of the cytostome, opening into the endoplasm. The lips of this orifice are formed by the pellicle and the ectoplasm. Outflow of the fluid endoplasm is no doubt prevented by a limiting membrane which appears to line the endoplasmic vesicle. This endoplasmic vesicle communicates with the oesophagus. Further research is needed to make clear the significance of this intercommunication. The writer was unable to differentiate in *Buxtonella sulcata* a co-ordinated neuromotor mechanism with fibres connected to a motorium as described by McDonald (1922) for *Balantidium coli*. However, in well stained whole mounts, and especially in longitudinal sections (see Pl. XXXII, figs. 8–12), a cone of long fibres is seen which surrounds the funnel-shaped oesophagus, cytostome and oral end of the peristome. The writer (1922) found similar fibres in *Paramaecium oecaudatum*.

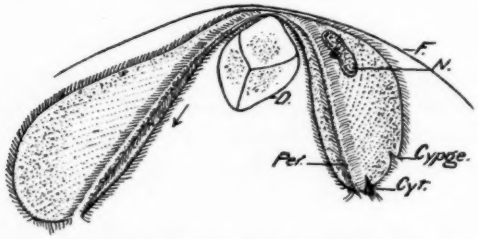
Cysts of *Buxtonella sulcata*.

Using the binocular microscope the writer found cysts in the normal faeces of infected cows. The directions of Sheather (1923) for a flotation technique were followed and by this means a number were collected. They differed from cysts of *Balantidium coli* in that, while the cysts were still viable, the nuclei were clearly visible through the walls. The writer was unable to confirm the finding of Becker and Hsiung (1929) that the indentation of Jameson (cytopyge of the writer) was visible in the cyst. About 12 hours after administering Epsom salts (1½ lb.) to an infected cow, active trophozoites appeared in the liquid faeces. In about 15 hours the consistency of the faeces began to approach the normal and the trophozoites contained therein were rounded up though ciliary motion was still in progress. About 2 hours later the forms that were passed were enveloped by a membrane, but within this coat the ciliary action was still manifest. At the end of another 2-hour period fully formed cysts began to appear. The rounded up trophozoites and the trophozoites that were active within the enveloping membrane were opaque, but, as stated above, the fully formed cysts became transparent.

Behaviour of *Buxtonella sulcata*.

Favourable material for the observation of living organisms was maintained at or near body temperature. When viewed under the 4 mm. objective or under an oil immersion the ciliate usually rotated on an axis through the region of the cytostome and perpendicular to the plane of the slide in either a clockwise or counter clockwise direction. Forward locomotion also occurred frequently and in every case the remarkable phenomenon of "swimming backwards," with the mouth to the rear, was verified. The above-mentioned rotation appeared to be due to the stronger beat of the cilia on one side of the body than on the other side, and not to a reversal of the effective stroke. It

continued as a rule for about 45 minutes, the direction changing every 10 or 15 minutes. Streaming of food particles through the cytostome into the endoplasm was noted, but food vacuoles did not appear to form. Jameson also was unable to note the formation of food vacuoles. No flow could be detected in either direction through the cytopyge. It is, of course, evident that the environment of the ciliate during these observations was far from normal. Ciliary movement became gradually slower until it ceased and several minutes later a small bulge of endoplasm through the cytostome occurred. Frequently small globules of endoplasm protruded through the cytostome during active ciliary movement and were pinched off to float free as spheroidal particles. Outstreaming of the endoplasm occurred soon after death, usually through the cytostome, but occasionally through a rupture of the ectoplasm and the pellicle. When individuals were placed in ink or dilute solutions of tannic acid or of mercuric chloride, the outstreaming occurred immediately in every case



Text-fig. 6. Sketch from memory of an individual of *Buxtonella sulcata* that was observed while passing through a very narrow aperture between the surface film of a drop on the cover glass and a particle of debris. *F.* surface film of the drop; *Cypge.* cytopyge; *Cyt.* cytostome; *N.* nuclei; *Per.* peristome; *D.* particle of debris.

through the cytostome. When the organisms were observed in larger quantities of faecal material under the binocular microscope, the swimming movements resembled those of *Paramaecium*, being in a spiral direction accompanied by rotation in a clockwise direction on the longitudinal axis. An observation illustrated in Text-fig. 6 suggests a method of inter-cellular tissue penetration by ciliates. This figure was drawn from memory, of an organism that was passing between an obstacle and the surface film of the liquid. As actually observed, the behaviour resembled strikingly that of the polymorphonuclear leucocytes when seen on a slide passing between the red blood corpuscles. McDonald (1922) observed and figured a similar phenomenon in *Balantidium* but made no comment concerning its significance. It is apparent that, like amoebae, ciliates may pass through very minute pores.

Viability.

The meagre observations thus far recorded appear to indicate that the trophozoites will disappear from the faeces in about 3 hours when this material is cooled to room temperature, and within 36–40 hours at 37·5° C. They differ

in this respect from balantidia which the writer (1927) maintained for 10 days at about 20° C. It is difficult, however, to compare viability by this method in such light infections as occurred in *Buxtonella sulcata* with heavy infections of balantidia in pigs. The cattle ciliates withstood manipulation with the pipette and under the cover glass much better than balantidia, as they lived for almost an hour as compared with less than 15 minutes for balantidia.

Notes on the morphology of *Balantidium coli*.

Balantidia from the pig were studied because it was at first thought that the ciliate of cattle described above was a member of the genus *Balantidium*. The morphological studies by McDonald (1922) were confirmed except in the following particulars: (1) an oral plug within the cytostome could not be differentiated either in fixed preparations or in living organisms; (2) a body could not be found that corresponded to the motorium described and figured by McDonald; (3) centralised neuromotor fibres were likewise not differentiated. Drawings of the first four of a series of 28 cross-sections cut at 3 microns (Pl. XXXII, figs. 13–16) do not show any of the above-mentioned structures. They indicate that the cytostome is at the bottom of a cone-shaped peristome and that in *Balantidium coli*, as in *Buxtonella sulcata*, the oesophagus, if ciliated, is thus specialised at only the extreme oral end (see Pl. XXXII, figs. 13–16). A cone-shaped zone of long fibres was found to surround the peristome, cytostome and oesophagus and to correspond to similar fibres that were described and figured by McDonald (see Pl. XXXII, figs. 17–20). As stated above, such fibres occur likewise in *Buxtonella sulcata*. In neither ciliate does the morphology suggest that they function in the co-ordination of stimuli. They appear rather to be myonemes. Both of the above Protozoa inhabit the lower portion of the intestinal tract and specialisation appears to be primarily physiological, adapting the organisms to life in an environment of complex biochemical reactions.

Summary.

1. A ciliate occurring in the caecum of about 25 per cent. of the cattle that were examined at Jeanerette, Louisiana, is herein classified as *Buxtonella sulcata* Jameson (1926) and it is proposed that it be removed from the Class Aspirigera, Family Isotrichidae, and included in the Class Spirigera, Order Heterotrichida.

2. The ciliate is characterised by the position of the cytostome near the permanently open cytopyge and within the peristome which continues from the cytostome around the oral end of the body to the extreme aboral end which during locomotion is anterior.

3. The infections were detected in slaughtered animals by examining faecal material in Syracuse watch glasses under the binocular microscope, this method of faecal diagnosis having advantages over the smear method.

4. By the administration of Epsom salts the ciliates may be obtained from living cattle.

5. A pipette is described and figured for the isolation of Protozoa.

6. Death of *Buxtonella sulcata* in an unfavourable environment occurred by disruption of the endoplasm through the cytostome.

7. *Buxtonella sulcata* disappeared from faecal material at room temperature more quickly than balantidia from the pig, but withstood manipulation under the microscope better than the balantidia.

8. The passage of an individual between two obstructions suggested a means of intercellular tissue penetration by ciliates.

9. The oral plug and the centralised neuromotor system described and figured by McDonald in *Balantidium coli* could not be differentiated by the writer, but McDonald's other morphological observations on the above ciliates were confirmed.

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EXPLANATION OF PLATE XXXII.

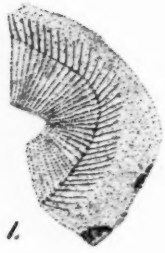
Figs. 1–12 of *Buxtonella sulcata*. Figs. 13–20 of *Balantidium coli*. All figures were drawn by camera lucida from sections cut at 3 microns and stained with iron haematoxylin. Figs. 1–7 (× 900) are drawings from a series which contained 38 cross-sections through one individual.

Buxtonella sulcata.

Fig. 1 is of section 38 that passed through the posterior end of the organism and shows a row of specialised body cilia and the parallel rows of basal granules of other body cilia. The extreme end of the reversed J-shaped bend of the peristome is also indicated.

Fig. 2 shows a row of adoral cilia extending across the peristome and also on its margins a continuation of the above-mentioned row of specialised body cilia.

Figs. 3 and 4 were drawn from sections 36 and 35 respectively which passed through the deepest part of the peristome leading to the cytostome. Note the continuation of the rows of adoral cilia. A groove to the left of the peristome is seen in Fig. 3 and is continued in Fig. 4, in which the pellicle and ectoplasm are broken through to form the cytopyge.



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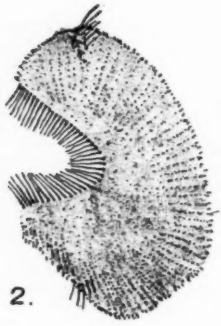

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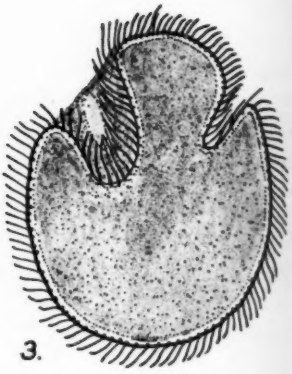

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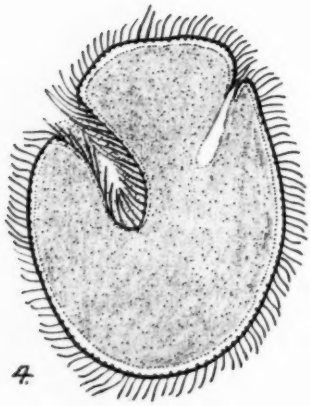

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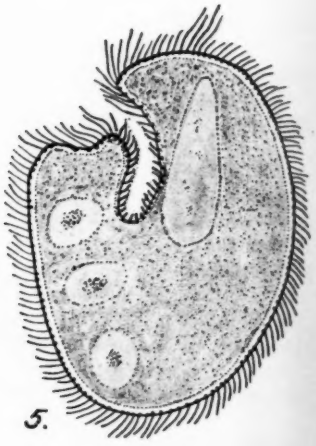

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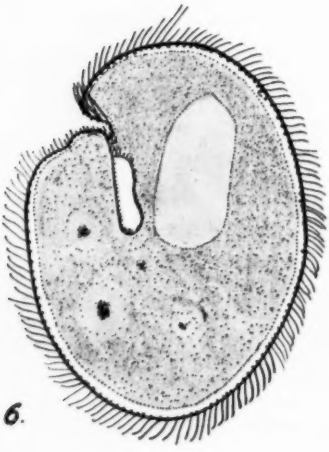

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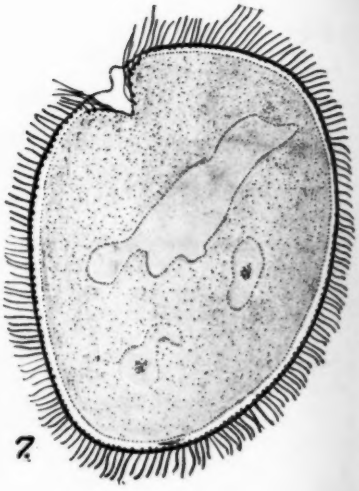

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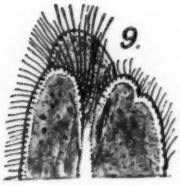
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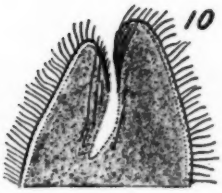
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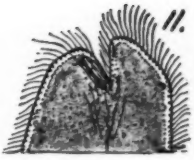
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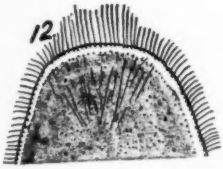
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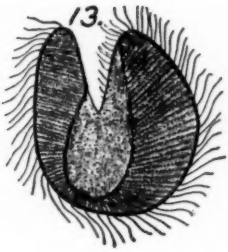
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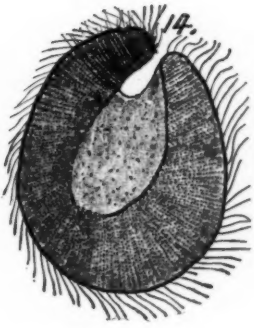
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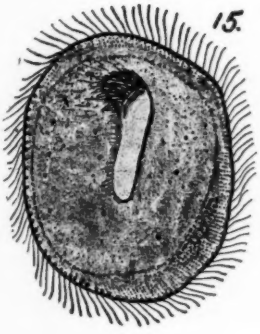
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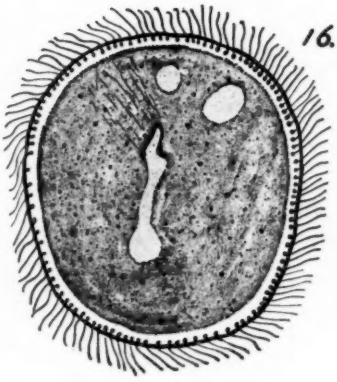
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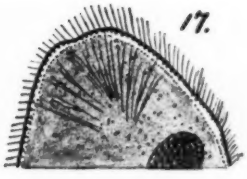
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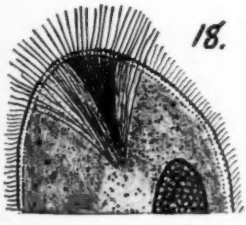
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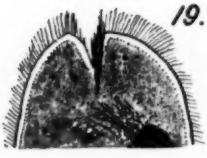
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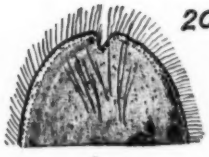
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Fig. 5 shows the cytostome, the margins of the peristome, and the continuity of the adoral and oral cilia, also a large vacuole within the endoplasm into which the cytopyge opens.

Fig. 6 shows the cytostome, also a section through the peristome and a continuation of the endoplasmic vacuole.

Fig. 7 shows the enlarged vacuole which permits intercommunication between the oesophagus and the cytopyge. The peristome, at this point, is a groove on the ventral surface of the body.

Figs. 8–12 (× 600) are of serial longitudinal sections through one individual. The series contained 21 sections. The margin of the groove of the cytopyge is seen in Fig. 8, also the neuromotor fibres. Fig. 9 shows the relationships between the peristome, cytostome and oesophagus, also of the adoral, oral and oesophageal cilia. Fig. 10 is of a section that passed longitudinally through the peristome, cytostome, and oesophagus, and also shows the cilia. The outer margin of the peristome and the adoral cilia are shown in Fig. 11, also the neuromotor fibres which are seen more distinctly in Fig. 12.

Balantidium coli.

Figs. 13–16 (× 1100) are of the first four of a series of 28 cross-sections through *B. coli*. Note the peristome, cytostome, and oesophagus, the oral, adoral and body cilia, and the thickened ectoplasm; the endoplasm is limited in this region to a narrow cone, the position of which is eccentric.

Figs. 17–20 (× 600) are of longitudinal sections through *B. coli* and show the neuromotor fibres surrounding the oesophagus, cytostome and peristome.

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THE MORPHOLOGY OF *ZALOPHOTREMA HEPATICUM*, WITH A REVIEW OF THE TREMATODE FAMILY FASCIOLIDAE.

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(With Plate XXXIII, including Figs. 1–4.)

The description of the species is made from seven specimens found in the liver of a California sea-lion, *Zalophus californianus*, which died in the Aquarium of the New York Zoological Society. The worms were referred to the Department of Biology, New York University, for study and identification by Robert T. Hatt, Assistant Curator of Mammals at the American Museum of Natural History, who made the dissection and to whom grateful acknowledgment is made. In a preliminary report (Stunkard and Alvey (1929)) a brief description of the parasites was given, and they were named *Zalophotrema hepaticum*. The name indicates the nature of the worms as well as the identity of their host. The type-specimen (Fig. 1) is deposited in the collection of the Department of Lower Invertebrates, American Museum of Natural History.

The worms are large, elongate, with parallel sides; flattened dorsoventrally, rounded anteriorly, and attenuated posteriorly. They vary from 11 to 13 mm. in length, 3 to 3·6 mm. in width, and 0·6 to 1 mm. in thickness. In the fixed specimens the anterior portion of the body is much thicker than the posterior region which contains the testes and vitellaria.

The acetabulum is spherical, 0·57 to 0·62 mm. in diameter, situated one-fifth to one-sixth of the body length from the anterior end.

The cuticular covering of the body measures from 0·004 to 0·007 mm. in thickness and is heavier on the dorsal surface. It is armed with large recurved spines which vary in length from 0·014 to 0·033 mm. Those near the anterior end of the body and around the acetabulum are largest, and they gradually decrease in size posteriorly. The spines are embedded in the cuticula, and not only project from the surface but extend through the cuticula, so that their bases rest in minute depressions in the dermo-muscular wall of the body. The body wall contains the usual circular, longitudinal and oblique muscle layers, and parenchymal fibres make connections with the internal organs. The cortical region of the body parenchyma has a nuclear zone that contains the bodies of cells which apparently secrete the cuticula and the spines. The mesenchymal tissue of the body is relatively compact and close-meshed.

The mouth opening is slightly subterminal, and the oral sucker measures from 0·7 to 0·8 mm. in length by 0·8 to 1 mm. in width. The oral sucker is followed almost immediately by the pharynx, a pyriform organ, with the narrower portion directed anteriad. It measures from 0·55 to 0·6 mm. in length, and from 0·38 to 0·42 mm. in greatest width. The digestive system bifurcates a short distance behind the pharynx, and the two tubes thus formed pass laterally and anteriorly. They are lined with cuticula for some distance, and in this respect agree morphologically with the oesophagus of other distomes. The oesophagus may accordingly be described as consisting of three regions, a very short median portion and two branches which extend laterally and communicate with the intestinal caeca. On either side, the caecum bifurcates to form anterior and posterior branches. Each of the anterior branches gives off two or three diverticula, and then turns mediad to terminate near the anterior end of the pharynx. The posterior caeca extend from the level of the pharynx to the caudal end of the body. They bear many diverticula, directed both laterally and medially throughout their length.

The excretory pore is situated at the posterior end of the body, and the excretory vesicle extends anteriorly as a simple sac to the region of the ootype. Tubules appear in sections, but it has not been possible to work out the details of the system in fixed and sectioned material.

The testes are large, irregular in shape, and deeply lobed, with the anterior lobes of each testis again subdivided. They lie one behind the other in the middle third of the body. The cephalic testis is situated immediately behind the ootype, and its anterior lateral lobes are crossed by the transverse vitelline ducts. A vas deferens passes forward from each testis. These ducts are dorsal in position, and at the posterior end of the cirrus sac empty into a large seminal vesicle. The cirrus sac extends on the dorsal side of the body from the genital pore to the level of the ovary. It has a thin fibro-muscular wall which contains circular and longitudinal fibres, although they are not developed into definite layers.

A seminal vesicle, the anterior portion of which is coiled, occupies the caudal three-fourths of the cirrus sac. The initial portion of the vesicle is lined with columnar epithelium, although the body of the organ has a low epithelial lining. From the seminal vesicle a narrow duct leads to the genital pore. Only the terminal portion, 0·012 to 0·018 mm. in length, is lined with cuticula (Fig. 4), and the rest of the duct is lined with columnar epithelium. The duct, throughout its length, is surrounded by prostate cells, whose bodies lie near the wall of the cirrus sac and whose ducts traverse a mesenchymatous zone around the central canal. The cirrus is probably eversible, although it was not observed in that condition. No spines are present on the terminal portion of the duct and the cirrus must be unarmed.

The ovary is a lobed, irregularly shaped gland, situated at the caudal end of the anterior third of the body. It is about 1 mm. in width and 0·5 mm. in anteroposterior extent. The lateral lobes are directed forward on either side of

the body. The oviduct arises from the dorsal posterior tip of the ovary as an expanded tube containing many large cells. It diminishes in diameter as it passes posteriad and dorsad, where it enters the ootype. Here Laurer's canal is given off, and the common duct from the vitelline receptacle is received. From the ootype the uterus passes forward on the dorsal side of the body, and this initial portion frequently contains spermatozoa. There is no seminal receptacle. The region of the ootype (Fig. 3) is surrounded by the cells of Mehlis' gland which lies dorsal and posterior to the ovary. Laurer's canal passes dorsally to open on the dorsal surface a short distance behind the ootype.

The vitellaria consist of masses of follicles, which extend in the lateral areas of the body from the level of the pharynx to the posterior end. They invest the branches of the intestinal caeca whenever possible, and in the anterior half of the body tend to be dorsal in position. The arrangement is probably due to the presence of other structures, acetabulum, uterus, ovary, and testes, which occupy the ventral area in this region. Behind the testes, the vitellaria are both dorsal and ventral, and in this region they invade the median field. Collecting ducts extend longitudinally along the median faces of the vitellaria, and the right and left ducts unite near the posterior end of the body. In one specimen there is a common median duct in the region behind the testes. At the level of the ootype, transverse ducts pass mediad on the dorsal side of the body, meeting in the median plane to form a vitelline receptacle. From the receptacle a common duct passes ventrally and opens into the ootype. The uterus, after its origin from the ootype, passes forward on the dorsal side of the ovary. In front of the ovary it forms masses of coils that fill the central part of the body to the level of the acetabulum. The metraterm is about one-half the length of the cirrus sac and lies directly beneath it. Coils of the uterus lie on both sides of the portion of the metraterm that extends behind the acetabulum. In the sectioned specimens both metraterm and cirrus sac open into a common sinus, and the genital pore is just anterior to the acetabulum.

The uterus is filled with thick-shelled eggs, each of which contains a solid mass of cells. The eggs (Fig. 2) are oval, with a distinct operculum at one end and a polar projection at the other. The opercular end is flattened and the operculum is thinner than the rest of the shell. In cross-section the eggs are circular. Eggs measure 0·068 to 0·079 mm. in length, and 0·043 to 0·052 mm. in width. The egg shell is 0·004 to 0·005 mm. in thickness, and the polar projection is from 0·006 to 0·007 mm. in length.

Discussion.

A comparison of *Zalophotrema hepaticum* with previously described forms shows close morphological agreement with *Fasciola* Linnaeus, 1758, *Fasciolopsis* Looss, 1899, and with the worms which have been found in the liver of various aquatic mammals and assigned to the genera, *Campula* Cobbold, 1858

(=*Brachycladium* Looss, 1899), *Lecithodesmus* Braun, 1902, and *Orthosplanchnus* Odhner, 1905. All of these genera were included by Odhner (1910) in the family Fasciolidae. He says (p. 164): "Was endlich diejenige Familie betrifft, die mit dem Namen *Fasciolidae* s. str. um den Prototypus der Distomen, *Fasciola hepatica* (L.), herum zu bilden ist, so scheint sich dieselbe aus drei Unterfamilien zusammenzusetzen: 1. *Fasciolinae* mit *Fasciola* L. s. str. und einer neuen Gattung für *Dist. magnum* Bassi, 2. *Fasciolopsinae* mit *Fasciolopsis* (Lss.) Odhn. und einer neuen Gattung für *Dist. tursionis* Marchi (= *D. longissimum* Poir.), 3. *Brachycladiinae* mit den Gattungen *Brachycladium* Lss. (? = *Campula* Cobb.), *Lecithodesmus* Brn. und *Orthosplanchnus* Odhn."

In an earlier paper Odhner (1905) had removed *Brachycladium* and *Lecithodesmus* from the sub-family Fasciolinae, and had included these genera, together with *Orthosplanchnus*, in a new sub-family Brachycladiinae.

Subsequent changes in the composition and organisation of the family Fasciolidae have not been numerous. In accordance with the suggestion of Odhner (1910), Ward (1917) erected the genus *Fascioloides* for the parasite from American herbivores which was named *Distomum magnum* by Bassi. Odhner (1926) restudied *Distomum robustum* von Lorenz from the intestine of the African elephant, a species which had been described also by Poirier as *Distomum kerandeli*, and concluded that it represented a prototype of the large liver fluke. For it he created the new genus *Protofasciola*.

In the paper cited earlier (Odhner, 1905), the differences between the subfamilies Fasciolinae and Brachycladiinae were listed. In his 1926 paper Odhner did not refer to the classification which he had previously proposed (Odhner, 1910), but reverted to his 1905 arrangement, and without further evidence raised these two groups to family rank. The name of the first group was changed to Fasciolopsidae, with *Fasciolopsis* as type, and in it were included the three genera *Fasciolopsis*, *Fasciola* and *Protofasciola*. It is apparent that actually what Odhner did was to suppress the sub-family Fasciolinae, raise the subfamily Fasciolopsinae to family rank, and transfer to it the genera formerly included in the Fasciolinae. Such procedure is not in agreement with the established rules of zoological nomenclature. The *International Rules of Zoological Nomenclature*, Article 5, state: "The name of a family or sub-family is to be changed when the name of its type genus is changed." In the case under consideration, the name of the type genus was not changed; instead, another genus was selected as type. If the action of Odhner were sustained and allowed to serve as a precedent, endless confusion would result. On such a basis, it would be possible for an author who believed that the type genus of any given family was not the most representative form to select another genus as type and change the name of the family. *Fasciola* was designated type of the sub-family and family to which it belongs, and if *Fasciola* and *Fasciolopsis* are to be included in the same group, one is obliged to recognise that *Fasciola* is the nomenclatorial type of the first available sub-family and family, and that the names of the corresponding groups must be Fasciolinae and Fasciolidae.

For the genera formerly included in the sub-family Brachycladiinae, Odhner (1926) proposed the family Campulidae, since, as had been contended by Braun (1900) and according to Odhner, and confirmed by the recent finding of Cobbold's original specimens in London, *Brachycladium* Looss is a synonym of *Campula* Cobbold.

No specific morphological differences were listed by Odhner (1926) as diagnostic for his two families, Fasciolopsidae and Campulidae. He called attention to the biologic difference, that members of the first infest terrestrial herbivorous and omnivorous mammals, whereas members of the second are parasitic in fish-eating marine mammals. It is of interest to note, however, that the life-history of none of these species from aquatic mammals has been determined, and consequently it is not at all certain that fishes are essential hosts in the life-cycle of the flukes. The bionomic distinction between the groups may therefore be less significant than believed by Odhner. Fuhrmann (1928) adopted Odhner's arrangement and used the characters of the previously outlined sub-families as diagnostic for the families. According to him, in Fasciolopsidae the excretory vesicle is much branched, the receptaculum seminis is absent, and the eggs are thin-shelled and circular in cross-section. In Campulidae, on the other hand, the excretory vesicle is sac-shaped, the receptaculum seminis is present although very small, and the eggs are thick-shelled and triangular in cross-section. An examination of these characters as they appear in the different genera shows much variation. According to Odhner (1926), *Protofasciola* has "eine grosse, einfach schlauchförmige Exkretionsblase" which communicates dorsally with a branched system of canals. Such a condition may be regarded as intermediate between those present in *Fasciola* and in *Campula*. The other points of difference listed by Fuhrmann are invalidated by the structure of *Zalophotrema*. In most morphological features this form agrees much more closely with *Campula* than *Fasciola*, yet it has no receptaculum seminis. The eggs are circular in cross-section and the structure of the copulatory organs is of the fasciolid rather than the campulid type. Comparison of the arrangement of the organ systems, or of the form and relative position of their component parts, shows much overlapping among the several genera. The morphological distinctions between the two families largely disappear, and the doubtful bionomic distinction does not appear adequate to separate the genera into two families. Actually the morphological differences between *Protofasciola*, *Fasciola*, and *Fasciolopsis* are as great as the differences between any one of these genera and certain of those assigned to the family Campulidae. It appears, therefore, that Odhner's (1910) arrangement, assigning all of these genera to a single family, is the most logical system, and that his (1926) modification was a step in the wrong direction.

In a recent revision of the family Fasciolidae Travassos (1929) has proposed the incorporation of four sub-families, as follows:

1. Fasciolinae Stiles & Hassall, 1898, with the genera
   Fasciola Linnaeus, 1758, and *Fascioloides* Ward, 1917.

2. Fasciolopsinae Odhner, 1911,
   Fasciolopsis Looss, 1899, and *Protofasciola* Odhner, 1926.
3. Brachycladiinae Odhner, 1911,
   Brachycladium Looss, 1899; *Campula* Cobbold, 1858; *Lecithodesmus* Braun, 1902; *Orthosplanchnus* Odhner, 1905; and *Trifolium* Travassos, 1922.
4. Omphalometrinae Looss, 1899,
   Omphalometra Looss, 1899; *Cathaemasia* Looss, 1899; and *Pulchrosoma* Travassos, 1916.

The first three sub-families in the system of Travassos agree almost entirely with the arrangement of Odhner (1910). Travassos has hesitated to recognise the identity of *Campula* Cobbold and *Brachycladium* Looss, although there seems to be no doubt on the subject. Furthermore, he included in Brachycladiinae the genus *Trifolium*, erected to contain a species from the intestine of birds. The morphology of the species is incompletely known, the excretory system has not been described, and its inclusion in the sub-family is admittedly tentative. Fuhrmann (1928) listed it in a group of genera of uncertain systematic position. A parasite of the intestine of birds rather than the liver of mammals, the morphological and bionomic differences between it and other members of the Brachycladiinae appear to preclude its presence there. If it should prove to have an excretory system like that of the Echinostomidae, it might well be transferred to that family.

The sub-family Omphalometrinae was erected by Looss (1899) to contain *Omphalometra* and *Cathaemasia*, genera which in certain features resemble the Fasciolinae. Odhner (1911, p. 523) removed *Omphalometra* to the family Lepodermatidae, synonymous with the family Plagiorchiidae. Later, Odhner (1926) reported that the excretory system of *Cathaemasia* is similar to that of the Echinostomes and, "Die Gattung muss deshalb als eine Aberrante vorläufig den Echinostomiden angeschlossen werden." Fuhrmann (1928) designated *Cathaemasia* as type of a new family. It appears extremely doubtful, therefore, whether *Omphalometra* and *Cathaemasia* are closely related genera or whether either may be included in the family Fasciolidae. The third genus, *Pulchrosoma*, included in the sub-family Omphalometrinae by Travassos, contains a single species which has an excretory vesicle with lateral diverticula and which lives in the abdominal cavity and air sacs of birds. In morphological features it agrees with Fuhrmann's diagnosis of the family Fasciolopsidae, although because of its habitat and essential anatomical agreement with the echinostomes it might equally well be referred to that group.

CONCLUSIONS.

Review of the morphology and bionomics of the fasciolid and related genera, and application of the rules of nomenclature, lead us to recognise the family Fasciolidae with three sub-families: (1) Fasciolinae Stiles & Hassall, with *Fasciola* as type and containing also the genus *Fascioloides*; (2) Fasciolopsinae

Odhner, with *Fasciolopsis* as type and containing also the genus *Protofasciola*; (3) Campulinae nom. nov., with *Campula* as type and containing also *Lecithodesmus*, *Orthosplanchnus*, *Zalophotrema*, and a new genus erected to receive the species *Distomum tursionis* Marchi (= *Dist. longissimum* Poirier).

Synthesium gen. nov.

Odhner (1910) predicated that *D. tursionis* represents a new genus of the sub-family Fasciolopsinae. As a result of later investigations he (1926) reported that the species manifests characters common to members of the Brachycladiinae: "es besitzt die überaus charakterischen Endapparate der Geschlechtswege von *Orthosplanchnus* Odhner, den Darmkanal von *Brachycladium* Looss und gelappte Hoden wie *Lecithodesmus* Braun." Tentatively the species was referred to *Orthosplanchnus*, although it is apparent that its inclusion there does violence to the morphological unity of the genus and cannot be accepted. For it we propose the new genus *Synthesium*.

SUMMARY.

A description of *Zalophotrema hepaticum* is presented. Study of this species and review of the literature show that the genera assigned to the two families, Fasciolopsidae and Campulidae, may be included in a single family. Furthermore, since *Fasciola* is the nomenclatorial type of the family to which it belongs, the rules of nomenclature do not permit the substitution of the name Fasciolopsidae for Fasciolidae. Consequently the family Fasciolidae is restored with three sub-families, Fasciolinae, Fasciolopsinae, and Campulinae. A new genus, *Synthesium*, is erected to contain *Distomum tursionis* Marchi.

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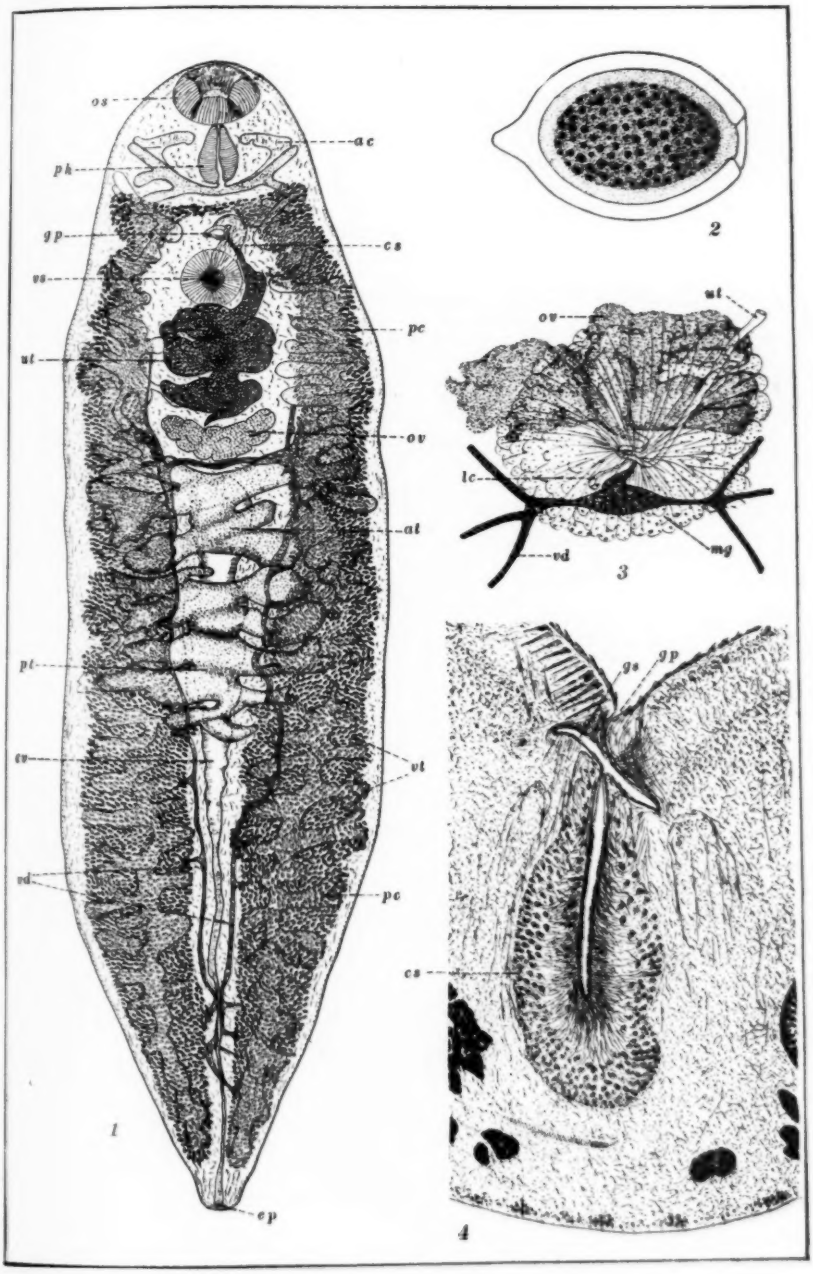
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EXPLANATION OF PLATE XXXIII.

Zalophotrema hepaticum.

Fig. 1. Type-specimen, ventral view.
Fig. 2. Egg.
Fig. 3. Female genital organs.
Fig. 4. Cirrus sac and genital pore.

Abbreviations: *ac*, anterior caecum; *at*, anterior testis; *cs*, cirrus sac; *ep*, excretory pore; *ev*, excretory vesicle; *gp*, genital pore; *gs*, genital sinus; *lc*, Laurer's canal; *mg*, Mehlis' gland; *os*, oral sucker; *ov*, ovary; *pc*, posterior caecum; *ph*, pharynx; *pt*, posterior testis; *ut*, uterus; *vd*, vitelline duct; *vs*, ventral sucker; *vt*, vitellaria.

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LERNAEA [*LERNAEOCERA*] *BARBICOLA* N.SP., A PARASITIC COPEPOD OF *BARBUS* SP. FROM THE TRANSVAAL.

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(With 6 Text-figures.)

Habitat and Record. By the courtesy of G. Evelyn Hutchinson of Yale University, I have received and examined a new species of *Lernaea* collected by him on 7. iv. 1928 on a small *Barbus*, which had lost all its scales and was too young for specific identification, from an alkaline pan in the Transvaal, S. Africa. For the purpose of comparison reference will be made to *L. elegans* described by me, *Parasitology*, 1925, **17**, 246.

Material. A single specimen ♀ embedded in the caudal myomeres of the host on the ventral side posterior to the anus (Fig. 1, *L*).

Body. The outline of the animal is best seen from Fig. 2. The total length of the specimen is 7·2 mm., of which the head plus neck occupies 2·3 mm., the trunk 3·4 mm.; while the ovisacs measure 2 mm. The animal is not of a green colour, and does not contain symbiotic algae.

The *head*, which is ovoid, bears anteriorly a spherical apex from which arise the appendages, and at the summit of which is the mouth. From the outer edges of the ovoid spring three *cephalic horns* (Figs. 3 and 4), two, paired, lateral and undivided, and one, unpaired, dorsal, broad, flattened and slightly notched, the usual chitinous organs of fixation of the Lernaeidae, in this case brown and not bluish. In this specimen the right lateral horn has a tendency to curl like a ram's: this is probably fortuitous. Owing to the disposition shown by all species of *Lernaea* to primary and even secondary dichotomy of the horns, it is difficult to predicate that the specific number of horns is three, for if the bifurcation of the dorsal horn be carried further with advancing age the number of horns will appear to be four. The fact that the paired horns are lateral and not ventral is a more reliable feature. Variability in the bifurcation of the horns within the same species has already been discussed by me (*Parasitology*, 1925, **17**, 250).

The *neck* is moderately short and stout and merges imperceptibly into the trunk without any posterior enlargement.

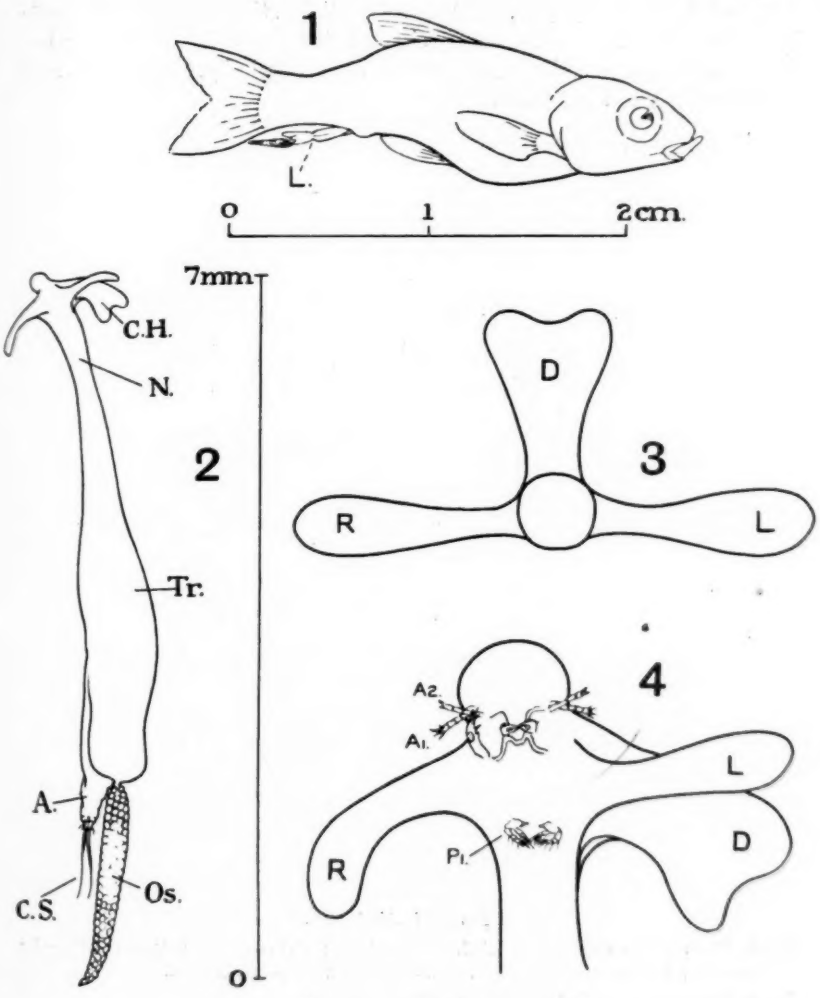


Fig. 1. *Barbus* sp. with attached parasitic Copepod, *Lernaea barbicola* ♀, n.sp. (*L.*).

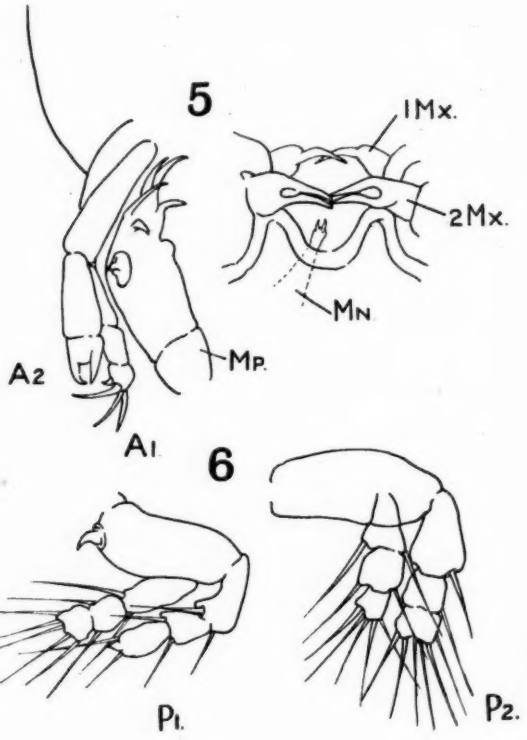
Fig. 2. *Lernaea barbicola* ♀, the entire animal with the anterior end in ventral aspect mounted on a slide in Farrant's medium. *C.H.* = cephalic horns; *N.* = neck; *Tr.* = trunk; *A.* = abdomen; *Os.* = ovisacs; *C.S.* = caudal styles.

Fig. 3. *L. barbicola*, plan of the cephalic horns from the anterior. *R.* = right lateral horn; *L.* = left lateral horn; *D.* = dorsal horn.

Fig. 4. *L. barbicola*, head in ventral aspect, enlarged view of Fig. 2. Lettering as in Figs. 3 and 5.

Appendages (all paired) are:

The 1*st Antennae* (Fig. 5, *A.* 1) are four-articled, the basal article being the longest and the others decreasing in size. The terminal article bears three long spinous curved setae apically, and a small one sub-apically. There are no setae present on any of the articles.



Lernaea barbicola n.sp.

Fig. 5. The anterior appendages. *A.* 1. = first antenna; *A.* 2. = second antenna; 1 *Mx.* = first maxillae; 2 *Mx.* = second maxillae; *Mn.* = mandible; *Mp.* = maxillipede.

Fig. 6. The pereiopods. *P.* 1 = first pair; *P.* 2 = second pair.

The 2*nd Antennae* (Fig. 5, *A.* 2) are three-articled and probably end in three finger-like processes, the middle one of which is absent in this specimen. There are no setae visible on any of the articles, of which the proximal is the longest and the others decrease in size.

The 1*st Maxillae* (Fig. 5, 1 *Mx.*) are one-articled, and uniramous and each ends in a single claw, exactly resembling that of *L. elegans*, but proportionately larger.

The *2nd Maxillae* (Fig. 5, 2 *Mx.*) consist each of a stout base which terminates in two movable claws. Both pairs of maxillae meet and overlap in the middle line.

The *Mandibles* (Fig. 5, *Mn.*) resemble those of *L. elegans*, but end in two spines instead of one.

The *Maxillipedes* (Fig. 5, *Mp.*) are large, indistinctly two-articled, the distal article being convex on the inner side, and terminating in five spines distributed as in *L. elegans*, but more feebly developed.

The **Trunk** (Fig. 2, *Tr.*) is moderately stout and the abdomen elongated. The ovisacs are proportionately longer than in *L. elegans*, and contain many rows of numerous eggs (Fig. 2, *Os.*). The abdominal appendages, on either side of a well-marked anus, take the form of caudal styles, each consisting of a minute two-articled base (the terminal article bearing one seta on its outer side) surmounted by an extremely long seta. These details resemble those of *L. elegans*.

The remaining thoracic appendages, usually miscalled "swimming legs," are herein termed *pereiopods*. All except the last pair of the five, as is usual, conform to type, each being biramous and strongly setigerous. Each ramus is three-articled and inserted on a common base (Fig. 6, *P.* 2). The base of the first pair bears proximally a well-marked incurved spine as in *L. elegans* (Fig. 6, *P.* 1). The fifth pair is difficult to distinguish, but each appears to be a rounded plate bearing three setae.

Specific characters ♀. Three cephalothoracic horns, paired, lateral, undivided, and one dorsal, broad, notched at apex. Neck moderately short and stout, not enlarged posteriorly. Abdomen elongated. Ovisacs long and slender. Maxillipedes terminating in five spines, two apical, and three sub-apical. Basal joint of 1st pereiopod with a strong incurved process toward the middle line.

Host: *Barbus* sp. (Transvaal).

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REPORT ON A COLLECTION OF CESTODA, MAINLY FROM EGYPT.

PART IV. CONCLUSION.

By F. J. MEGGITT.

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(With 2 Text-figures.)

Order **PSEUDOPHYLIDEA** Carus, 1863.

Family AMPHICOTYLIDAE Nybelin, 1920.

Sub-family Amphicotylinae Lühe, 1902.

Eubothrium Nybelin, 1920.

753. **E. crassum** (Bloch, 1779).

Host. *Salmo salvelinus.*

Family CARYOPHYLLAEIDAE Leuckart, 1878.

Originally containing only four genera, this family of recent years has been increased by the addition of twelve more and has further been subdivided by Hunter (1927, 1929) into four sub-families: Caryophyllaeinae, Lytocestinae, Pseudolytocestinae[1], Wenyoninae. Of these, the Lytocestinae and Pseudolytocestinae have vitellaria either wholly or partly in the cortical parenchyma and differ from each other only in the degree to which these are cortical; a difference hardly of sub-family rank, which should consist of characters of absolute not relative value: the Caryophyllaeinae and Wenyoninae, with vitellaria wholly medullary, differ from each other in the position of the genital pore and the absence of uterine glands, differences again not of sub-family rank. There remain therefore two well-marked sub-families, the Caryophyllaeinae and the Lytocestinae, the differences between which are given in the following table.

	Genital pore	Vitelline glands	Uterine glands
Caryophyllaeinae	Posterior quarter	Medullary	Present
Lytocestinae	Posterior quarter	Cortical	Present
Pseudolytocestinae	Posterior fifth	Partly cortical	Present
Wenyoninae	Anterior half	Medullary	Absent

The following tables give the differences, according to the diagnoses, of the genera of this family.

[1] Later changed to Capingentinae.

Caryophyllaeinae.

	Genital pore	Openings (♂ and ♀)	Scolex	Extent of uterine coils	External vesicula seminalis
Archigetes Leuckart, 1878	Posterior quarter of body	Into utero-vaginal canal	2 bothridia	Anterior to cirrus-sac	Present
Biacetabulum Hunter, 1927	,,	,,	2 suckers	,,	,,
Caryophyllaeides Nybelin, 1922	,,	,,	Undifferentiated	,,	Absent
Caryophyllaeus Müller, 1787	,,	No utero-vaginal canal	,,	Not anterior to cirrus-sac	,,
Glaridacris Cooper, 1920	,,	,,	6 loculi	,,	Present
Hypocaryophyllaeus Hunter, 1927	,,	,,	6 poorly developed loculi	Anterior to cirrus-sac	,,
Monobothrium Diesing, 1863	,,	,,	6 grooves + terminal introvert	Not anterior to cirrus-sac	,,
Wenyonia Woodland, 1923	Anterior half	—	—	,,	—

Lytocestinae.

	Scolex	Openings (♂ and ♀)	Vitellaria	Extent of uterine coils	Post-ovarian vitellaria
Balanotaenia Johnston, 1924	—	—	Wholly cortical	—	—
Capingens Hunter, 1927	2 bothridia	In genital atrium	Partly cortical	Anterior to cirrus-sac	Present
Djombangia Bovien, 1926	—	—	Wholly cortical	—	—
Lytocestoides Baylis, 1928	Furrows	In genital atrium	Wholly cortical (?)	—	Present
Lytocestus Cohn, 1908	Grooves	Separate	Wholly cortical	Not anterior to cirrus-sac	Absent
Monobothroides Fuhrmann & Baer, 1925	Numerous grooves + terminal introvert	,,	,,	,,	Absent
Pseudolytocestus Hunter, 1929	Undifferentiated	In genital atrium	Partly cortical	,,	Absent
Spartoides Hunter, 1929	6 loculi	Separate	,,	Anterior to cirrus-sac	Absent

Considering the genera of the first, *Biacetabulum* differs from *Archigetes* only in the presence or absence of a terminal excretory bladder and, if the latter be an adult form, in the presence or absence of a caudal vesicle: in view of the fact that the life histories of both are unknown, this latter difference cannot be admitted and, the remaining one being barely of specific importance, *Biacetabulum* becomes a synonym of *Archigetes*. *Hypocaryophyllaeus* and *Glaridacris* differ from *Monobothrium* in the degree of development of the scolex grooves. The scolex in this order is highly variable (cf. Fig. 1) and cannot be admitted as a character even of specific importance, except in such genera as *Duthiersia* or *Bothridium*, where it is permanently modified into a characteristic organ. The presence or absence of a vesicula seminalis and the degree of

the extent of the uterine coils again are not generic distinctions. These two genera, therefore, lapse into synonyms of *Monobothrium*. Similarly, as Woodland (1926, 61) has previously observed, *Caryophyllaeides* cannot be adequately distinguished from *Caryophyllaeus*. There remain four genera with well-marked characters. In the Lytocestinae, *Monobothroides* is synonymous with *Lytocestus*. The descriptions of *Balanotaenia* and *Djombangia* are inaccessible in Rangoon. Thus out of the fourteen genera, nine are valid according to the opinion of the writer. The writer is in agreement with the remarks of Woodland, "some efficient check is needed to prevent the absurd multiplication of genera which is nowadays taking place in most groups of the animal kingdom. The majority of systematists appear to consider it praiseworthy to elevate almost any character or combination of characters to the importance required for the founding of a new genus, and to view with equanimity or even satisfaction the creation of as large a number of genera as possible in any particular group." The tendency to exaggerate minor characters and to found families and genera on a single species with but few specimens is a retrograde step of dubious wisdom, resulting in reliance for specific distinctions upon differences hardly recognisable and of doubtful constancy. The cases of such genera as *Moniezia*, *Inermicapsifer* and *Phyllobothrium*, where species recently have been reduced to less than half, point the moral.

Sub-family CARYOPHYLLAEINAE Nybelin, 1920.

Caryophyllaeus Müller, 1787.

724. **C. laticeps** (Pallas, 1781).

Host. *Abramis brama*.

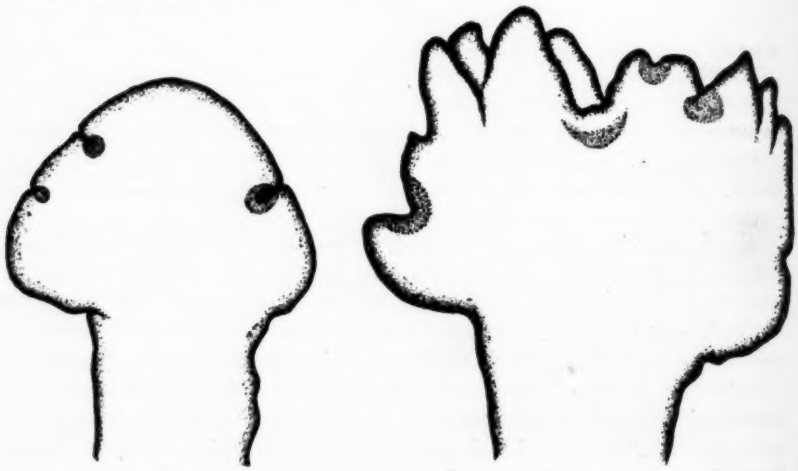

Fig. 1. *Caryophyllaeus laticeps*, two forms of scolex.

Scolex much broader than body, sometimes arrow-shaped, sometimes much folded and appearing as though possessing suckers. From the scolex two thick lateral muscle bands lead posteriorly: in the centre is a third, dividing again into two one-fifth of the way down the body, each half again subdividing. All bands disappear at the posterior two-thirds of the body length. Genital pore at posterior seventh of body. Testes extending from genital pore to anterior fifth of body length. Vitellaria form a sheath round the testes, ceasing —except for a small posterior group—anteriorly to the ovary. Uterine coils posterior to genital pore.

Sub-family LYTOCESTINAE Hunter, 1927.

Lytocestus Cohn, 1908.

741, 792. **L. filiformis** (Woodland, 1923).

Host. *Mormyrus* sp.

All specimens immature. In some two feebly developed bothridia were present.

Family BOTHRIOCEPHALIDAE E. Blanchard, 1849.

Bothriocephalus Rudolphi, 1808.

775. **B. claviceps** (Goeze, 1782).

Host. *Anguilla* sp.

Numerous fragments, all immature but most showing the anlagen of genital organs. Testes in oldest segments 43–48 each side, in younger 23–25; this number is much in excess of that previously recorded from this species.

Polyonchobothrium Diesing, 1854.

682, 730, 816. **P. clarias** (Woodland, 1925).

Host. *Heterobranchus anguillaris* (*Clarias lazera*).

Scolex 0·28–0·3 mm. dia. Hooks of scolex, 36, 0·034 mm. long, in two groups of 18 each, the separation being marked by a clear space dorsally and ventrally. Shape as figured: those described by Woodland (1925, 720) are probably not lying quite flat. Otherwise as described by that author. Mature proglottides absent.

Fig. 2. *Polyonchobothrium clarias*, rostellar hooks.

The species was placed by Woodland in the genus *Clestobothrium* by reason of its dilated uterus, distinct segmentation and lack of a neck, the armature of the scolex being considered of but secondary importance. The presence or absence of distinct external segmentation and neck cannot be considered as characters of much value, depending largely upon the preservation and state

of contraction of the specimens under observation. Thus, in the present case a neck is sometimes present, sometimes absent: external segmentation is regular but faint and does not correspond with the internal, either one or two sets of genital organs may be present in one apparent proglottis. In the Bothriocephalidae are five genera, distinguished, as in the following table, by the natures of the scoleces and of the uterus.

	Scolex	Armature	Uterine cavity	Receptaculum seminis
Bothriocephalus Rudolphi, 1808	Elongated, bothridia shallow	Absent	Present, not occupying whole proglottis	Absent
Clestobothrium Lühe, 1899	Spherical, bothridia sunken below surface	Absent	Present, occupying whole proglottis	Present
Polyonchobothrium Diesing, 1854	Elongated, bothridia shallow	Present	Present, occupying whole proglottis	—
Ptychobothrium Lünnberg, 1889	Arrow-shaped, bothridia well developed	Absent	Present, occupying whole proglottis	Present
Taphrobothrium	Elongated, bothridia shallow	Absent	Absent, uterus coiled	Absent

Taphrobothrium is clearly separated from the remainder by its winding uterus, devoid of an enlargement, and the position of the vitellaria. If Woodland's view, *i.e.* "the innumerable modifications of this type of scolex possess no more than a specific value in more detailed classification," p. 723, if this include the hooks as by the classification it appears to do—be adopted, *Clestobothrium* and *Ptychobothrium* become synonyms of *Polyonchobothrium* which, in its turn, is only separated from *Bothriocephalus* by the greater development of the uterus. If, on the contrary, scolex characters be considered as of generic value, then each becomes a distinct genus. In general, the armature of the scolex has been extensively used for the separation of genera, the shape of the hooks separating the Davaineidae, and the number of rows of hooks characterising *Porogynia*, *Anomotaenia* being separated from *Choanotaenia* by the double row of hooks, *Echinorhynchotaenia* being distinguished by its additional armature, the Onchobothriidae divided from the Phyllobothriidae by the armature of the scolex, etc.: the only cases where forms with different scoleces are included in the same genus are *Hymenolepis* and *Taenia*. While admitting that, in the past, the importance of the scolex has been greatly exaggerated, it is not necessarily without value (Lühe, 1900, 94), and to admit such a principle would reduce the present classification of the Cestoda to chaos. The presence or absence of definite hooks, as distinguished from spines, is a character of at least generic value, and may be used to distinguish

the genus *Polyonchobothrium*. Woodland (1924, 441) has described *Bothriocephalus pycnomerus*, a form with an armed scolex. Its inclusion in the genus *Bothriocephalus* is dubious. Should the uterine cavity comprise practically the whole proglottis—as, by the figure given (xxvii, 13) seems to be the case, the extreme shortness of the proglottides indicating their comparative immaturity—this form should be transferred to *Polyonchobothrium*, otherwise a separate genus is necessary should the degree of extension of the uterus still be held as of generic value. The following table gives the differences between the species of *Polyonchobothrium*, those between *P. cylindraceum major* and *minor* being of specific rank. It may also be remarked that the discrepancies between the descriptions of Klaptocz, on the one hand, and Diesing and Leydig on the other, suggest the presence of two distinct species, for the former of which the name *P. pseudopolypteri* is proposed.

	No. of hooks	Arrangement	Size (μ)	No. of testes	Extent of cirrus-sac	
P. armatum Fuhrmann	28	—	136	40–45	1/4 proglottis	
P. clarias (Woodland, 1925)	32–36	—	33–51	20	1/4 „	
P. cylindraceum major Janicki, 1926	32	4 groups	32–41–32	30	1/5 „	
P. c. minor Janicki, 1926	32	4 „	27–39–27	10–12	1/4 „	(?)
P. fulgidum n.sp.	32	1 row	11	12	2/3 „	
P. polypteri (Leydig, 1853)	24	4 groups	24–50	60	2/3 „	
P. pseudopolypteri n.sp.	32	4 „	100–150	60	—	
P. pycnomerus (Woodland, 1925) (?)	68	4 „	29–73–29	30–40	1/2 „	

784 *a*. **P. fulgidum** n.sp.

Host. *Heterobranchus anguillaris*.

Locality. Cairo.

Length 15 mm., breadth 0·1 mm. Scolex 0·13–0·2 mm. dia. Hook ring 0·1–0·12 mm. dia. Hooks approximately 32, in a single row, each 0·011 mm. long, of the usual shape. External segmentation faint. Genital pore at anterior quarter of proglottis margin, median. Cirrus-sac extending two-thirds of proglottis width. Testes 12, lateral to female glands. Ovary triangular. Eggs large. The above assemblage of characters does not agree with those of any species so far described.

Family TRIAENOPHORIDAE Nybelin, 1920.

Triaenophorus Rudolphi, 1793.

732. **T. nodulosus** (Pallas, 1781).

Host. ?

Locality. Leipzig.

A few strobilae, all immature. Assigned to this species by reason of the head being continuous with the body. Hooks 0·09–0·1 mm. from point to point. The differences between *T. nodulosus* and *T. robustus* Olsson, 1893, do not seem to be of specific value.

Trypanorhyncha Diesing, 1863.

Coenomorphus Lönnberg, 1889.

813. **C. grossus** (Rudolphi, 1819).

Host. *Mustelus vulgaris*.

A single specimen, 15 mm. long, 9 mm. broad. Scolex 9 mm. broad, 10 mm. thick, with two flat bothridia on opposite sides, 4 × 4 mm. On anterior border of each bothridium two small proboscides, close together and only slightly projecting.

SPECIES UNIDENTIFIABLE.

760.

Host. *Lamna* sp.

Only one decayed fragment seen in midst of whole mass of intestinal debris and portions of cork.

701.

Host. *Totanus calidris*.

Only a few fragments in a very poor state of preservation. Impossible to identify even the genus.

APPENDIX.

The following species were inadvertently omitted from their correct systematic position.

655. **Anomotaenia mollis** (Volz, 1900).

Host. *Gyps fulvus* (*Vultur fulvus*).

419. **Hymenolepis** sp.

Host. *Spatula clypeata*.

Locality. Marg.

All specimens immature and devoid of scoleces.

676. **Hymenolepis** sp.

Host. *Anas boschas fera*.

Locality. Nile.

Immature specimens devoid of scoleces. Apparently the same species as 419.

722. **H.** sp.

A few scoleces and anterior proglottides. Hooks and genital organs absent.

657 *b*, 691 *a*, 713, 790 *a*. **H. urceus** (Wedl, 1855).

Host. *Pelecanus onocrotalus* (657 *b*): *Platalea leucorodia* (691 *a*, 713, 790 *a*).

Length 2 mm., greatest breadth 0·37 mm. Scolex 0·21–0·25 mm. dia. Rostellum 0·03 mm. long, often, when extruded, with bulbous apex, sheath extending posteriorly to suckers. Rostellar hooks in two rows, 0·085–0·095 mm. and 0·12 mm. long (Krabbe 0·1–0·11 mm. and 0·073–0·076 mm.), similar in

shape to those figured by Krabbe (1869, Pl. iv). Proglottides comparatively few, much wider than long, except the square posterior ones. Genital pore unilateral, slightly anterior. Cirrus-sac 0·072–0·094 mm. (average 0·087 mm.) × 0·021–0·026 mm., extending $\frac{1}{3}$—$\frac{1}{2}$ across segment. Testes all in the same straight line. Eggs comparatively few.

RECTIFICATION OF NOMENCLATURE.

Hymenolepis furcifera Meggitt, 1927, and *H. pauciovata* Meggitt, 1927, are antedated by species proposed by Krabbe, 1869, and Fuhrmann, 1906: the names *H. flaminata* and *H. floreata* respectively are substituted.

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(*MS. received for publication*, 22. III. 30.—Ed.)

TROMBICULA FLUI N.SP., A PATATTA-MITE ("PATATTA-LUIS") OF SURINAM.

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University of Leyden.

(With 2 Text-figures.)

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I. Historical survey.

Since the description by Linnaeus in 1758 of the very irritating patatta-mite ("patatta-luis") of Surinam under the name *Acarus batatas*, this parasite has often been mentioned in the literature, but seldom have attempts been made to learn more about it. Furthermore, many important data have been omitted by authors in their writings, so that it seems desirable to summarise what is known of this mite.

Oudemans (1913) failed to obtain the patatta-mite in Paramaribo and his paper adds nothing to what Linnaeus had written of the mite. Oudemans, however, suggested that this mite might be identical with the well-known *Microthrombidium tlalzahuatl* of Mexico, or closely related thereto.

In 1911, Oudemans described *Microthrombidium helleri* from one specimen taken in June from *Passalus* sp. (one of the Lamellicornia) by C. Heller. Afterwards he stated: "Es ist möglich dass diese Species die berüchtigte batatas ist, denn sie ist sehr nahe verwandt mit der Mexikanischen tlalzahuatl."

Max von Brunn, in 1923, obtained material, said to have been caught on man, from the Missionary Station, Potriba, east of Paramaribo. This material was examined by Oudemans (1927) who identified the mites as *M. helleri* and concluded that his species was identical with *Acarus batatas* L. I cannot, however, agree with Oudemans as to the names *M. helleri* and *M. batatas* being synonyms, because the latter applies to a doubtful species (see further, p. 354). I therefore retain the name *helleri* in this paper.

Professor Flu, Director of our Institute, whilst in Surinam in 1927, collected patatta-mites in October and November. He did this by having some farmers stand with bare feet and legs in grassy land, where it was known that these mites occurred; at once the mites came creeping up the men's legs and they were caught in a tube, in the bottom of which was cotton-wool with ether. The mites were next transferred to 70 per cent. alcohol. He succeeded thus in collecting a number, and the material was kindly handed over to me for examination.

As the following description shows, these mites are not identical with *Microthrombidium helleri* Oudemans and *M. tlalzahuatl* Oudemans (which should now be called respectively *Trombicula helleri* and *Tr. irritans*; see p. 352), but they evidently belong to an undescribed species to which I give the name of *Tr. flui*[1], after the collector. After describing this species I shall point out the differences between it and other well-known American *Trombicula* larvae.

II. Description of the larva of *Trombicula flui* n.sp.

When unengorged, the body is almost round; measuring, whilst unfed, 169μ from the tip of the chelicera to the posterior margin of the body, and 113μ in width. Capitulum width (except palpi) 46·8μ. Colour reddish, when alive, according to Prof. Flu.

Body. *Dorsal surface.* Scutum (Fig. 1 *a*) punctate, trapezoidal, anterior margin slightly convex, posterior strongly convex, sides slightly concave, anterior and posterior angles broadly rounded; measuring sagittally 41·4μ and 68·4μ in width. Pseudostigmata round, situated a little behind the middle of the scutum, nearer to the middle line than to the sides.

Ocular plate elongate, smooth, with four nearly round eyes, the anterior pair (Fig. 1 *b*) well developed, the posterior pair (Fig. 1 *c*) smaller (according to Dr Oudemans the eyes are larger than in *helleri*). Pseudostigmatic organs (Fig. 1 *d*) filiform, 46μ long, slender, ending pointedly, in the distal half probably pinnated in two rows. Other scutal setae thicker, rod-shaped, both posterior ones blunt, ending a little less bluntly than the dorsal abdominal setae; anterior hairs more pointed. Anterior scutal setae always directed posteriorly, never anteriorly as Oudemans figures in his numerous larvae. Except the two collateral anterior setae which are more pinnated than the others, the scutal setae are pinnated in two rows, with a few short lateral barbs in the dorsal setae behind the scutum.

Dorsal setae distributed in five transverse rows of successively 10, 8, 8, 4, 4 setae (on one side I found an extra seta between the third and fourth row, which forms as it were a sixth row); these setae are obtuse at the apex with 10 to 12 short lateral barbs on the two (probably) dorso-lateral sides.

Ventral surface. Coxae punctate, each with one pectinate seta. Coxae I somewhat elongate, not triangular, with clearly undulated inner margin as in

[1] In conformity with the most accepted views at the present time, I do not use the generic name *Microthrombidium* Haller, 1882.

helleri; coxae II elongate, coxae III shorter than coxae II. Stigmata rather big. Setae on coxae II smaller and less pectinate than those on coxae I and III. Between coxae I and between coxae III a pair of pectinate setae, pointed

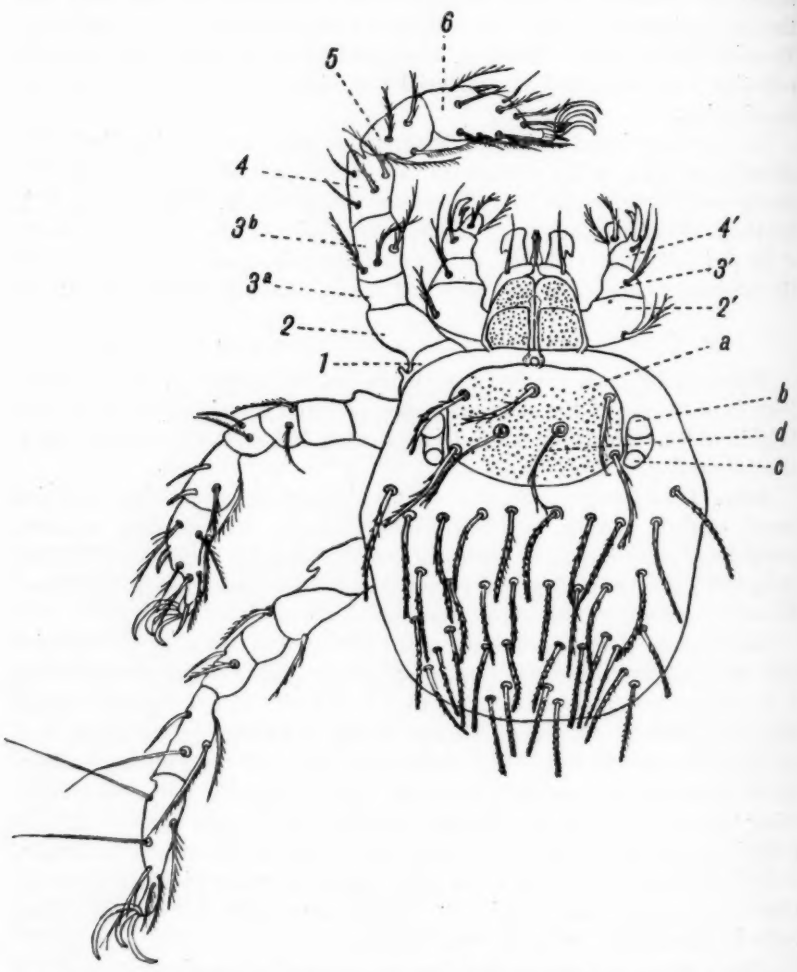

Fig. 1. *Trombicula flui* n.sp., dorsal aspect. *1.* coxa, *2.* trochanter, *3a.* basifemur, *3b.* telofemur, *4.* genual, *5.* tibi, *6.* tarsus. *2'*, *3'*, *4'*, palpal articles. *a.* scutum, *b.* anterior eye, *c.* posterior eye, *d.* pseudostigmatic organ.

at the tip, which are more delicate than dorsal setae. Behind coxae III abdominal setae distributed in five transverse rows of successively 4, 3 (on one side only 1 seta), 2, 4, 4 setae; on terminal border of the larva one row of

2 setae. Setae of the first three rows pointed at the apex, the other setae obtuse. Uroporus field narrow elliptical.

Capitulum. *Dorsal surface.* Mandibles form together an almost equilateral triangle (less pronounced than in *helleri*) with a strongly convex fore-part and somewhat rounded angles. Chelicerae long with an apical backwardly directed

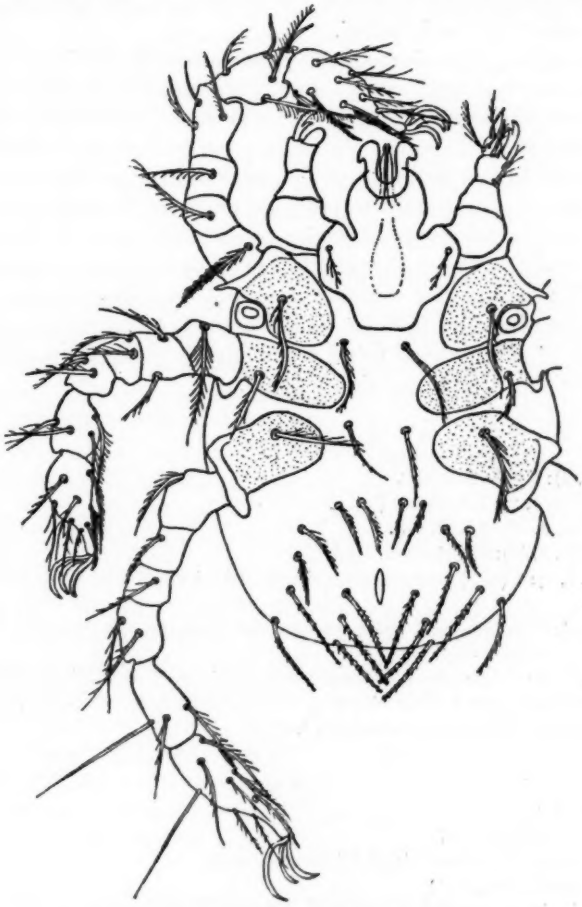

Fig. 2. *Trombicula flui* n.sp., ventral aspect.

tooth dorsally. Maxilla-galeae show clearly a more chitinised and a transparent membranous part. First part broad, anteriorly flat and bearing exteriorly a long simple seta somewhat behind the flattening. Dr Oudemans wrote to me, that this seta, in his opinion, seemed to be of another nature than in *helleri*; here it resembles an olfactory seta. Transparent membranous part rounded anteriorly and showing exteriorly a hooked notch.

Palpi thick; trochanterofemur (second segment; Fig. 1, *2'*) bears proximally externo-laterally a seta with some 8 barbs; genual (third segment; Fig. 1, *3'*) bears dorsally a seta with 2 outwardly directed barbs; on tibia (fourth segment; Fig. 1, *4'*) dorsally 1 simple seta, on appendiculum (thumb) 2 barbed setae, of which 1 is situated more laterally. Papal claw strong, straight and two-pronged, ventri-internal accessory prong certainly as sharp as major prong, without rounded apex as in *helleri*.

Ventral surface. Posterior part of hypostome differently formed in various individuals, now regularly convex, then with narrow end, because the sides converge rather strongly backward half way along their length. Hypostome median, anterior to the palpi, but slightly constricted, as in *helleri*; in the middle broadened and then constricted rather suddenly at the level of palpal trochanterofemur. Behind the palpi hypostome bears on each side a very fine seta with only a few barbs. Anterior membranous part U-shaped, partly hiding the shafts of the chelicerae. Second palpal segment strongly crooked on the inside; tibia bearing ventrally 1 barbed seta, appendiculum ventrally 2 simple setae of which 1 is situated more laterally and 3 barbed setae.

Legs. Legs short, stout, pair I being slightly stronger than the others. Articles bearing pectinate and simple setae[1].

Chaetotaxy as follows[1]:

Leg I.

Coxa (Fig. 1, *1*): 1 pect. (ventral).
Trochanter (Fig. 1, *2*): 1 feathered (v.).
Basifemur (Fig. 1, *3a*): 1 pect. (v.).
Telofemur (Fig. 1, *3b*): 4 pect. (3 d., 1 v.).
Genual (Fig. 1, *4*): 2 (or 3) nearly as long, simple (2 d., 1 v.); 4 pect. (2 d., 1 v., 1 externo-lateral).
Tibia (Fig. 1, *5*): 2 nearly as long (ventro-externo-lateral), simple; 7 (or 8) pect. (2 d., 3 interno-lateral, 1 externo-lateral, 1 or 2 v.).
Tarsus (Fig. 1, *6*): 1 short, conical sensory and one simple (dorsal, distal), besides at the terminal lobate part a short simple seta of another, narrower type; 17 pect. (5 d., 2 externo-lateral, 4 interno-lateral, 6 v.).

Leg II.

Coxa: 1 pect. (v.).
Trochanter: 1 feathered (v.).
Basifemur: 2 pect. (1 externo-lateral, 1 interno-lateral).
Telofemur: 4 pect. (2 d., 2 v.).
Genual: 1 thin simple seta (d.); 3 pect. (1 d., 1 externo-lateral, 1 v.).
Tibia: 2 simple, of which the distal one a little broader and somewhat different in structure; 6 pect. (1 d., 1 externo-lateral, 2 interno-lateral, 2 v.).
Tarsus: 1 short, conical sensory seta and on the terminal lobate part a short simple seta of the same type as on tarsus I; 14 pect. (4 d., 1 externo-lateral, 3 interno-lateral, 6 v.).

[1] Abbreviations used: d. = dorsal, v. = ventral, pect. = pectinate seta or setae.

Owing to movability of the legs it is difficult to state precisely if certain setae are situated laterally, dorsally or ventrally, consequently but a limited significance should be ascribed to the positions of setae, in this respect, that are given in the description that follows.

LEG III.

Coxa: 1 pect. (v.).
Trochanter: 1 pect. (externo-lateral).
Basifemur: 2 pect. (1 externo-lateral, 1 interno-lateral).
Telofemur: 2 (or 3) pect. (1 d., 1 v., sometimes another externo-lateral).
Genual: 1 simple seta (d.); 3 pect. (1 d., 1 externo-lateral, 1 v.).
Tibia: 1 simple seta as on the other articles and 2 very long, straight, outwardly protruding plain setae; 4 pect. (1 d., 2 interno-lateral, 1 v.).
Tarsus: 3 plain setae, very long, straight, protruding outwards (2 d., 1 v.); 11 pect. (4 d., 3 interno-lateral, 4 v.).

All tarsal claws rather stout and distinctly curved, empodium longer than claws and more slender.

III. DIFFERENCES BETWEEN *TROMBICULA FLUI* AND OTHER AMERICAN *TROMBICULA* LARVAE.

It is necessary to compare the descriptions of American *Trombicula* larvae, including those prevailing outside Dutch Guiana, especially as Verdun and Mandoul maintain that the patatta-mite also occurs in other parts of Central America, viz. the Antilles and Brazil. But we are not sure that this information is correct. According to a personal communication by Dr P. H. van der Hoog, one-time Chief Medical Officer of the Hygienic Service in Curaçao, patatta-mites are not found on that island. They certainly do not occur in New Guinea as maintained by the above-mentioned authors.

Trombicula helleri (Oudemans, 1911).

This mite differs from *Trombicula flui* as follows:

Scutum 60 × 103μ; front margin straight, posterior to the pseudostigmata a pair of transparent spots; the 3 anterior scutal setae directed anteriorly. Setae on the dorsum distributed in five transverse rows of successively 6, 6, 4, 4, 2 setae, abdominal setae (behind coxae III) in five transverse rows of successively 4, 2, 2, 2, 2 setae. Palpal trochanterofemur bears a seta with only a few barbs, genual bears a simple seta, tibia bears ventrally a simple backwardly bent seta; accessory prong of palpal claw short with rounded apex. Posterior part of hypostome bears on both sides behind palpi a beautiful barbed seta. For further chaetotaxy of legs, see table.

Trombicula tlalzahuatl Ewing, 1923 (*Microthrombidium tlalzahuatl* Oudemans, 1911).

Scutum 60 × 90μ; the 3 anterior scutal setae directed anteriorly. Palpal thumb bearing 2 simple and 5 barbed setae. Setae on dorsum distributed in five rows of successively 6, 6, 4, 4, 2 setae, abdominal setae (behind coxae III) in four transverse rows of successively 4, 2, 2, 2 setae. Posterior part of hypostome bears on both sides behind palpi a beautiful barbed seta. For chaetotaxy of legs, see table.

Chaetotaxy of legs compared:

	Tr. helleri		*Tr. tlalzahuatl*		*Tr. flui*	
	Simple seta	Short sensory seta	Simple seta	Short sensory seta	Simple seta	Short sensory seta
Genual I	.	1	1	1	2 (3)	.
Tibia I	.	1	.	.	2	.
Tarsus I	1	.	1	1	1 (short)	1
Genual II	1	.	1	.	1	.
Tibia II	1	.	2	.	2	.
Tarsus II	2	.	1	1	1 (short)	1
Genual III	.	.	1 (long)	.	1	.
Tibia III	.	.	1 (long)	.	2 (very long) + 1	.
Tarsus III	1 (long) + 1 (very long)	.	2 (long)	.	3 (very long)	.

Further we have to pay some attention to *Leptus irritans* and *L. americanus* Riley, 1873, because the patatta-mite has been called by this name by Prof. Flu in his report on his travels in Surinam, and because Verdun and Mandoul's text-book as well as Mense's text-book and other works on tropical hygiene and parasitology all state that these mites occur in central and meridional America and so possibly in Surinam.

Leptus americanus Riley, 1873. Genus ?.

When comparing Riley's figure of *L. americanus* with that of *Tr. flui* one can see at once that they belong to different genera. With good reason Ewing (1925) stated that "it can now be fairly definitely stated that *americanus* is not a species of Trombidiidae at all, but is rather a species of the family Erythraeidae, a group to which the genus *Leptus* really belongs, as Riley's figure clearly shows."

Leptus irritans Riley, 1873
(= *Trombicula irritans* Ewing, 1925).

During many years it was not known which mite Riley had observed, but when Ewing (1925) had the opportunity of studying all the specimens of *L. irritans* in Riley's collection, he found that they were identical with *Tr. tlalzahuatl*; therefore this mite must be called *Tr. irritans*, which is distinct from *Tr. flui*.

It is easier to compare *Tr. flui* with the other American harvest mites[1] because generally there exist good descriptions of them.

The chief points of difference between them and *Tr. flui* are as follows:

Tr. bruyanti (*Microthrombidium bruyanti* Oudemans, 1910).

Mandible claws without teeth; strongly barbed seta on trochanterofemur; externi-ventral prong of palpal claw needle-shaped; tarsus III bears one long simple seta.

[1] It is confusing to call these animals "chiggers" as this name has been given to the sandflea *Dermatophilus penetrans*.

I have not been able to consult the article in which Ribeyro and Bambaren described the *Leptus japa* reported by Escomel.

Tr. splendens Howard, 1918.

Size 250 × 170 μ; scutum bears three pairs of barbed setae, except the two pseudostigmatic filiform organs; a pair of barbed setae anteriorly to the eyes; setae on dorsal and ventral surfaces and on the palpi differently arranged. Chaetotaxy of legs neither figured, nor described. Anterior membranous part of hypostome not U-shaped.

Tr. dentata Ewing, 1925.

Palpal claw with three prongs at tip ; a row of backwardly directed teeth on the upper margin of the chelicerae.

Tr. irritans var. *tropica* Ewing, 1925.

Very similar to *Tr. irritans*, but the first palpal seta typically with one long barb near the tip and slightly below it a short barb.

Tr. panamensis Ewing, 1925 and *Tr. insularis* Ewing, 1925.

Papal claw very unequally divided, the inner prong being larger than the outer.

Tr. hylae Ewing, 1925.

Palpi rather slender, three-pronged palpal claw; scutum small, about as long as broad; the shield with a median angular process behind.

Tr. brasiliensis Ewing, 1925.

Closely resembles *Tr. flui*, as to scutum, structure and chaetotaxy of palps, but differs as follows: (*a*) in size (unengorged 200 × 160 μ), (*b*) abdomen with fourteen pairs of dorsal setae arranged into four irregular transverse rows as follows, beginning with the most anterior row: 6, 8, 8, 6; below the abdomen bears nine pairs of setae, an anterior row of 6, followed by a transverse row of 4, then a single pair near the median line, a transverse row of 4 setae and a posterior single pair of setae near the median line.

Tr. brumpti Lahille, 1927.

Size 270 × 180 μ; scutum slightly concave at front margin, slightly convex at posterior margin, setae on dorsum distributed in five transverse rows of successively 4, 8, 2, 6, 6 setae, on the ventral side (behind coxae III) in six transverse rows of successively 2, 2, 6, 2, 2, 2 setae. Tibia of palp seems to bear one barbed seta.

Tr. yorkei Sambon, 1928.

The shape of the scutum may be described as formed by the opposite halves of two concentric circular discs united diametrically, the anterior semicircle being twice the size of the posterior one and the latter ending caudad with a crescentic rim of thickened chitin.

IV. THE NAME "PATATTA-MITE."

It may be that the name "patatta-mite" is a collective term, including both *Tr. helleri* and *Tr. flui*.

As yet we only know that *Tr. flui* has been found on man (I found blood in the stomachs of some specimens). Oudemans' *helleri* material consisted of 100 specimens of this mite and many particles of scratched-off brown skin of an undetermined kind, *Amblyomma pseudoconcolor* Aragão, *Dermatophilus penetrans* and a rodent louse *Hoplopleura* sp. (as Oudemans informs me). The host or hosts of this mixed lot of parasites is problematical although material

from man was asked for. It must also be remembered that *Tr. helleri* was first collected from a beetle, whereas, as far as I know, mite larvae parasitic on man are not found on insects.

I do not suppose that the patatta-mite occurs on man only, but since Linnaeus described his species as man-derived, we can at present only call those mites patatta-mites which have actually been caught on man.

Only when it has been proved by further research that *Tr. helleri* does, in fact, live on man, shall we be able to say with certainty that the name "patatta-mite" is a collective name, including *Tr. flui* and *Tr. helleri*.

Oudemans assumes that *Acarus batatas* Linn. is identical with *Tr. helleri*, but for the reasons I have advanced I consider his conclusion premature, neither can I as yet regard *Tr. flui* as identical with *A. batatas*, as this might give rise to unnecessary confusion.

In an article which will be published shortly[1] the pathological significance of the patatta-mite will be discussed, as also its connection with the etiology of *Leishmaniosis americana*.

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[1] See *Geneeskundig Tijdschrift voor Ned. Indië*. An English translation will appear in *Acta Leidensia*, v.

(*MS. received for publication*, 7. iii. 1930.—Ed.)

ORNITHODORUS PAPILLIPES BIRULA AND *O. CHOLODKOVSKYI* N.SP.

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(With Plate XXXIV and 17 Text-figures.)

O. papillipes (Birula, 1895).

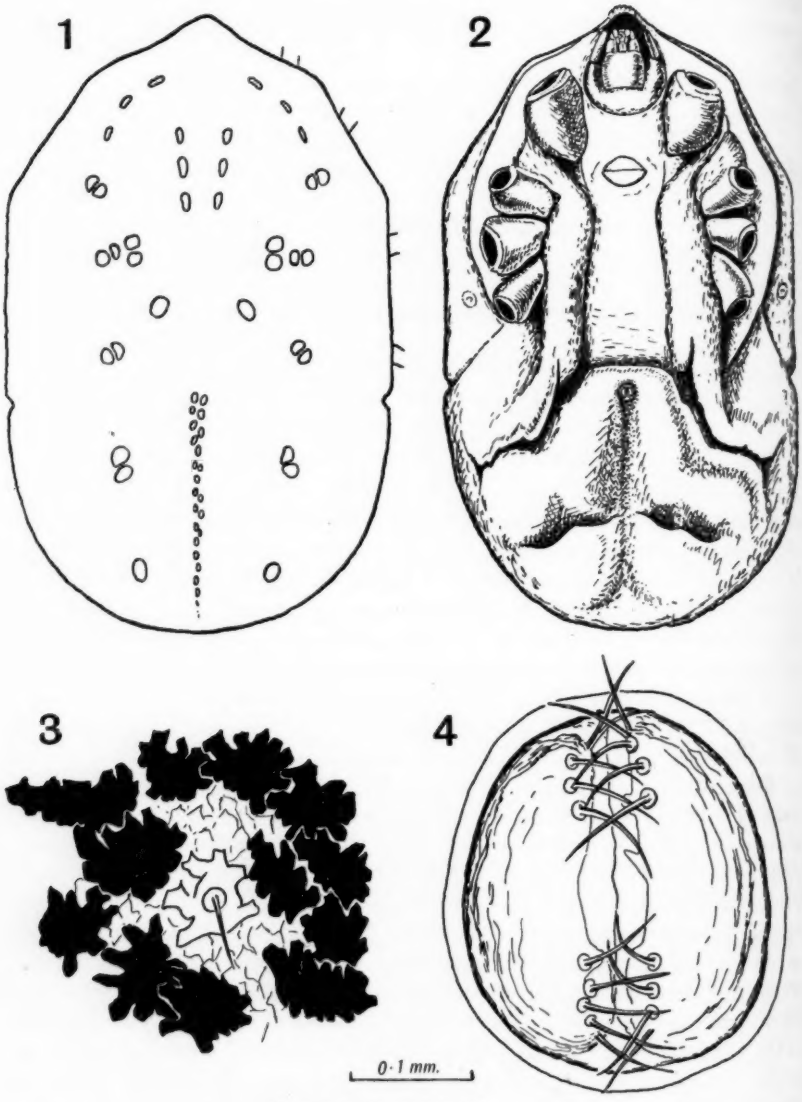
Synonymy: *Argas papillipes* Birula, 1895.
Ornithodorus crossi Brumpt, 1921.

(Pl. XXXIV, figs. *A–C*. Text-figs. 1–11.)

I have examined (*a*) alcoholic and microscopic specimens of Birula's type-material of *O. papillipes* preserved in the Zoological Museum of the Academy of Sciences, Leningrad, and (*b*) hundreds of living ticks from different localities in Turkestan which I determined as *O. papillipes*. The larger number (*b*) were chiefly supplied by Dr I. A. Moskwin, who, in my laboratory and at the Infectious Diseases Clinic of our Academy, demonstrated that the spirochaete of Turkestan, relapsing fever, is transmitted by this tick.

A more detailed account of this species appears desirable, in view of the extensive material at my disposal and the greater knowledge of the systematics of ticks which has been acquired since Birula's time.

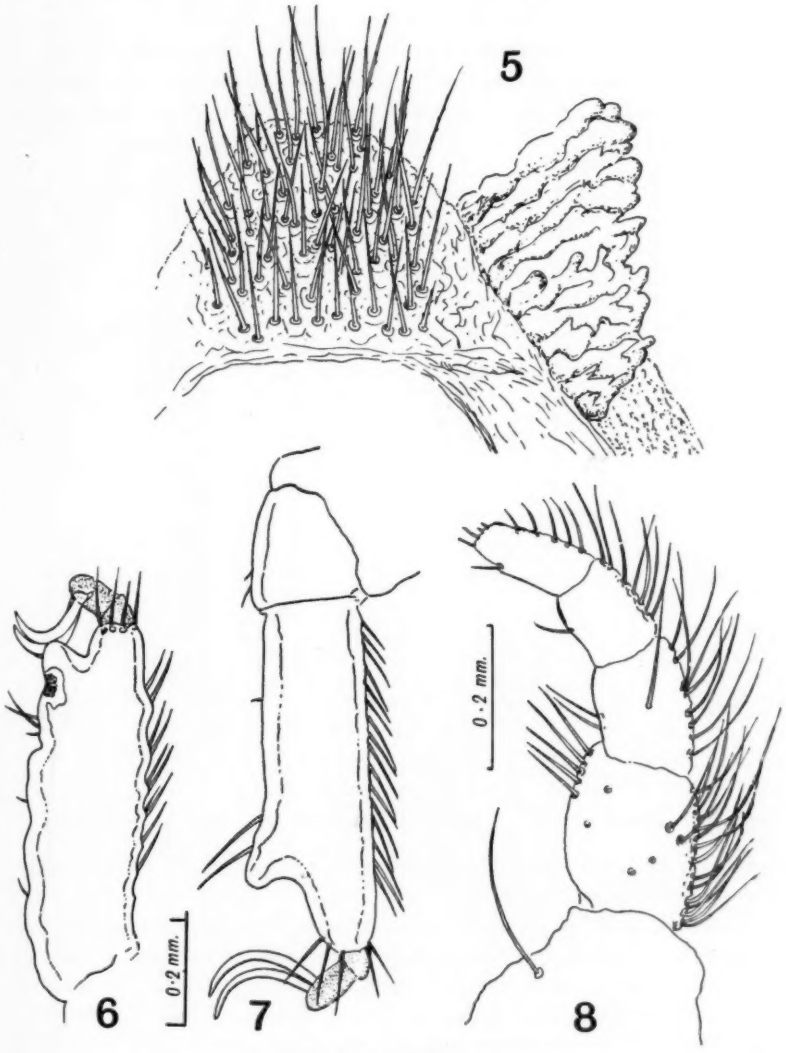
Female. Body dark grey, form as in Pl. XXXIV, figs. *A* and *B*, the size, varying with the state of engorgement, being 7·8 × 4·6 to 8·2 × 4·6 mm. The arrangement of the dorsal discs is shown in Text-fig. 1; corresponding depressions occur in unfed ticks. The cuticle shows a reticulate structure with irregular protuberances interspersed, these being largest marginally; it appears wrinkled in unfed specimens; the centre of each mesh of the network pattern appears, as shown in Text-fig. 3 (highly magnified), to be occupied by a single hair. Text-fig. 2, from an unfed specimen, shows the arrangement of the furrows on the ventral surface and the sides and anterior part of the body, also the position of the vulva, anus and spiracles. Eyes absent. The anal ring measures 25 × 23 to 31 × 25 microns, each anal plate bearing 9–10 hairs situated as shown in Text-fig. 4. The vulva is 54–62·4 microns wide. The capitulum lies in a camerostome with posterior and lateral margins protruding, the latter terminating where the slightly movable "cheeks" begin; the cheeks (sclerites) are coarsely wrinkled and humpy. Text-fig. 5 shows the structure and chaetotaxy (denticulated hairs) of the ventral body surface which protrudes beyond the capitulum. The basis capituli measures 63–65 microns. Text-fig. 8 shows the



Figs. 1–4. *O. papillipes* ♀.

Fig. 1. ♀. Distribution of discs on dorsum.
Fig. 2. ♀. Ventral surface.
Fig. 3. ♀. Structure of Chitin on dorsum, the blackened areas denote plates in the new work—the white central plate is depressed and bears a hair.
Fig. 4. ♀. Anal valves.

anterior portion of the basis and the lateral view of the palp with its fine smooth hairs. Hypostome with almost parallel sides and dentition as shown in Text-fig. 9; two long hairs arise at the sides of the hypostome base as in all



Figs. 5–8. *O. papillipes* ♀.

Fig. 5. ♀. Showing left sclerite of camerostome and hairs arising from the conical antero-ventral surface of body in front of the camerostome.

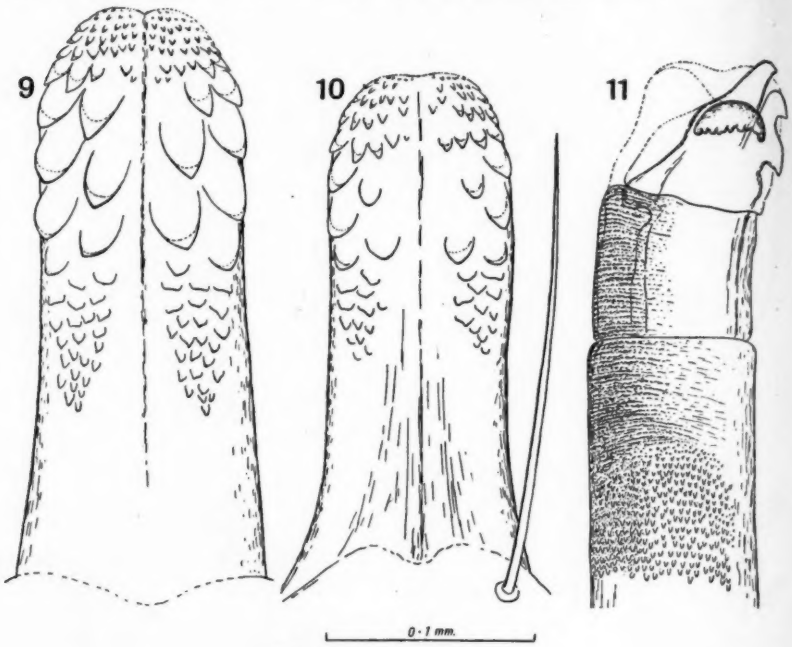
Figs. 6 and 7. ♀. Tarsi I and IV.

Fig. 8. ♀. Palp.

argasidae. The structure of the sheath and digit of a chelicera are shown in Text-fig. 11. Coxa I large, distant from II; coxae II–IV closely grouped. Tarsus I (Text-fig. 6) with wavy dorsal contour, Haller's organ elongate oval. Tarsus IV as shown in Text-fig. 7

Male. Size 4·2 × 2·5 to 5·8 × 3·4 mm., genital aperture facing coxae I, hypostome (Text-fig. 10) shorter than in female and with smaller teeth.

Habitat. *O. papillipes* was first recorded from the Caucasus; it has been found living in the cracks of clay walls in stables and human dwellings in

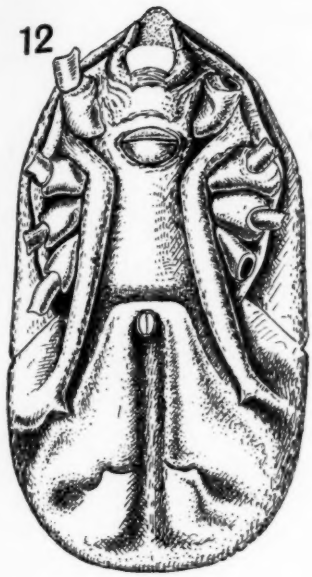


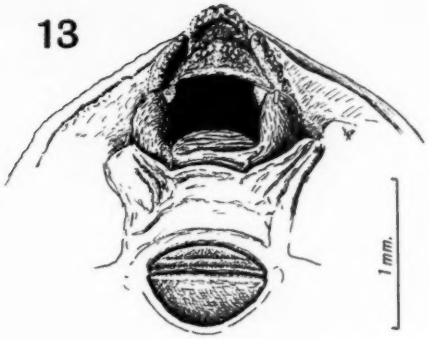
Figs. 9–11. *O. papillipes* ♂♀.

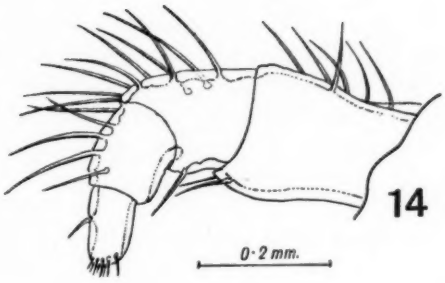
Fig. 9. ♀. Hypostome. Fig. 10. ♂. Hypostome. Fig. 11. ♀. Chelicera.

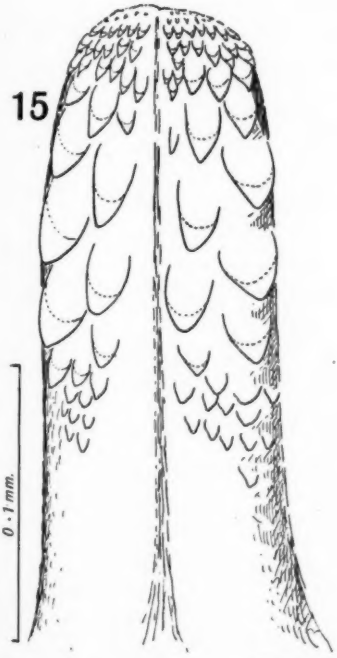
Turkestan. *O. crossi* Brumpt was discovered by Capt. H. E. Cross in native stables, north Punjab, India.

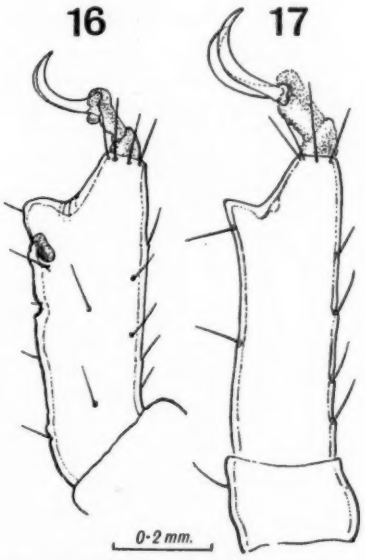
Systematic position. Nuttall and Warburton (1908, p. 79) regarded *O. papillipes* as a doubtful species, no type material being available to them for examination; they, however, give a brief description of the main characters and reproduce the original figures of Birula. Specimens identified by me as *O. papillipes*, after comparison with Birula's type-material, were sent by me for examination to Nuttall and Warburton in Cambridge. These specimens were found by them to be identical with *O. crossi* Brumpt, 1921. They sent these specimens on to Prof. Brumpt in Paris, and he also agreed that my specimens and *O. crossi* were one and the same species. Therefore, in accordance with the rules of priority, the name *O. crossi* falls into synonymy.











Figs. 12–17. *O. cholodkovskyi*, ♀.

Fig. 12. Ventral surface. Fig. 13. Camerostome and vulva.
Fig. 14. Right palp, external surface. Fig. 15. Hypostome.
Figs. 16–17. Tarsi I and IV.

O. cholodkovskyi n.sp.

(Pl. XXXIV, fig. *D*, Text-figs. 12–17.)

Female. Body relatively slenderer than in *O. papillipes*, size 7·4 × 3·7–4·2 mm. Viewed ventrally (Text-fig. 12) the coxal folds bend outwardly very markedly between coxae I and II. Vulva 0·8 mm. wide. The camerostome "cheeks" but slightly developed, the bulging thereof being most marked laterally (Text-fig. 13). The ventral surface of the anterior conical protrusion of the body bears no hairs (the contrary in *O. papillipes*, see Text-fig. 5), and the palps (Text-fig. 14) show different relative lengths of the articles, beside bearing fewer hairs than in *O. papillipes* (compare Text-fig. 8). Hypostome (Text-fig. 15) much like that of *O. papillipes* (Text-fig. 9). Tarsus I (Text-fig. 16) recalls that of *O. papillipes* but it is hardly papillate. Tarsus IV (Text-fig. 17) with more pointed dorsal spur and fewer hairs (compare with Text-figs. 6 and 7). Finally it differs in the structure of the cuticle.

This species is based on one female taken by A. Gerassimow in Turkestan, and named in honour of my late master, Prof. N. A. Cholodkovsky.

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EXPLANATION OF PLATE XXXIV.

Figs. *A* and *B*. *Ornithodorus papillipes* ♀, dorsal and ventral aspects.
Fig. *C*. *Ornithodorus papillipes* ♂, dorsal aspect.
Fig. *D*. *Ornithodorus cholodkovskyi* ♀, ventral aspect.

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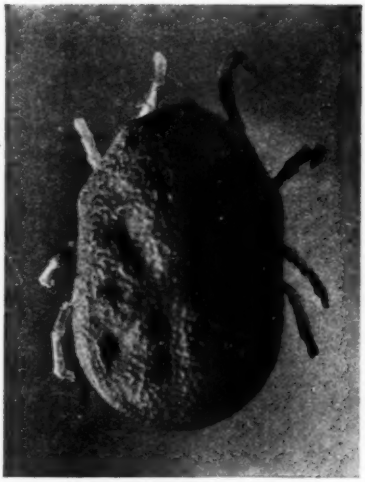

A

B

C

D

UNIV OF MICH

BAIRAMLIA FUSCIPES WATERSTON, A CHALCIDOID PARASITE ON *CERATOPHYLLUS WICKHAMI* BAKER (SIPHONAPTERA).

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(With 4 Text-figures.)

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I. Introduction.

During October 1928 I had occasion to dissect a number of cocoons of the flea, *Ceratophyllus wickhami*, from the nest of a North American Grey Squirrel (*Sciurus carolinensis*) from Gerrard's Cross, Buckinghamshire. In a few of the cocoons I found fully fed larvae or pupae of a Chalcidoid parasite, together with the empty skin of the flea larva. Among the nest material several minute wasps were crawling about, which proved to be the adults of the parasitic larvae in the cocoons.

On several occasions nests of Grey Squirrels from Tonbridge (Kent) were also examined, but no parasites were found. The fleas in these nests were *Ceratophyllus sciurorum*.

Dr J. Waterston (1929) has recently described the adult parasite which he found to represent a new genus of the Pirinene tribe of the family Miscogasteridae. He called it *Bairamlia fuscipes*. This is the first parasitic Hymenopteron to be found parasitising a flea.

It is impossible to say whether *Bairamlia fuscipes* is a native of this country, as the Grey Squirrel and the flea *Ceratophyllus wickhami* came originally from North America. It seems unlikely, however, that when the squirrel came to England, it brought this parasite with it, in addition to its own specific flea.

II. Bionomics.

Owing to the scarcity of parasites in the squirrel's nest, it has not been possible to breed a supply of them in the laboratory, though attempts have been made with some satisfactory results. The number of parasites found during fifteen months is shown in Table I. A certain quantity of material from the nest at Gerrard's Cross was sorted whenever available, and all the flea cocoons (if any were present) were dissected, and frequently the flea larvae also.

Table I. *The number of* B. fuscipes *found in* 15 *months, in material from nest of a Grey Squirrel from Gerrard's Cross.*

Month	Larvae	Pupae	Adults	Number of flea cocoons found and dissected
October 12th, 1928 ...	7	5	15*	120
„ 25th ...	0	3	3†	42
November 9th ...	1	0	0	50
February 12th, 1929 ...	3	4‡	0	25
March 27th ...	0	0	0	36
April 29th ...	0	0	0	12
May 4th ...	0	1	0	34
	Squirrels deserted the nest in May			
May 31st ...	0	2	0	10
June 19th ...	0	0	0	25
July–September ...	No material taken.		Squirrels returned in September	
October 15th‖ ...	0	0	2§	10
November 5th‖ ...	0	0	0	2
December 12th‖ ...	0	0	0	0
	11	15	20	366

26 cocoons parasitised—or 7 per cent. of the 366 cocoons dissected

* Males and females. † 2 males, 1 female. ‡ Dead. § Males. ‖ Nest material very wet.

Various methods have been tried to obtain conditions in which the parasite will breed. When fully fed Chalcidoid larvae were found in flea cocoons, these pupated and emerged if they were kept in gelatine capsules in a moist atmosphere, but the adults were often deformed and only lived a few days as a rule. The adult parasites can be kept with flea larvae and cocoons in boxes made of plaster of Paris, with a glass lid, but the best method for all stages is to keep them in small Petri dishes covered with fine muslin. These were either kept at the temperature of the laboratory or at the temperature of the insect house, which was not heated, and varied with the temperature of the day. The Petri dishes were always kept in desiccators over sulphuric acid and water in the proportion to give a relative humidity of 80 per cent., the humidity in which this particular species of flea larva was found to flourish with dried blood as food. A raisin was kept in the Petri dish also, as food for the adult parasites. The adult *Bairamlia fuscipes* is positively heliotropic, and can therefore be collected from a squirrel's nest material by putting the latter in a light-proof tin box, with a cork in the lid and a test tube through this as the only source of light. After a few days the parasites begin to appear in the test

tube. The adult parasites are very active, walking about quickly though never flying. They frequently walked over flea larvae, but these wriggled so violently that the parasites were frightened away. It seems unlikely that *B. fuscipes* ever oviposits on active flea larvae, and no eggs or larvae of the parasite were ever found on dissection of these. This appears to be the same in certain other Chalcidoids (Graham-Smith, 1915). The adult parasites paid more attention to flea cocoons, and tapped them with their antennae as though sensing the position and stage in development of the flea.

The parasites paired readily in captivity, but only on one occasion did they parasitise the cocoons given to them. On this occasion two female parasites, which emerged from cocoons on May 22nd and May 31st respectively, were put in a Petri dish with flea larvae and cocoons, which had been reared in the laboratory. On June 6th these flea larvae and cocoons were examined and dissected to see if they had been parasitised parthenogenetically, but there were no Chalcidoid eggs or larvae to be found. Parthenogenesis occurs in closely allied genera, for example, in *Pachyneuron*, a Pteromalid (Haviland, 1922) and in many other Chalcidoids (Parker and Thompson, 1928). A male parasite emerged on June 6th, and was put with the same two females in a Petri dish with a fresh supply of flea larvae and cocoons. These were observed carefully, and pairing took place between the male and one of the females on the day that the male emerged. The next day the female *Bairamlia* was seen paying particular attention to a certain cocoon, feeling it with her antennae and walking backwards and forwards across it. After nearly half-an-hour she inserted her ovipositor into the cocoon several times during about eight minutes. She then walked away and remained on a dead leaf for a long time. It was not possible to watch the three parasites much during the next few days. Six days later one female and the male *Bairamlia* died. The other female had had her wings chewed off, possibly by the flea larvae, and she died three days later.

Eight days after the female had been seen to pierce the cocoon, the flea cocoons and larvae from the Petri dish were dissected. The cocoon in which the parasite had been observed to insert her ovipositor contained a resting flea larva, apparently alive, but completely immobile. On the fourth abdominal segment there was an opaque patch with an apparent puncture in the middle, but no larva or egg could be found internally or externally. It seems probable that the parasite had injected some paralysing fluid, but not oviposited. Of the remaining six cocoons, three had been parasitised. The first resting larva had a small Chalcidoid larva firmly fixed on the dorsal surface of the fourth abdominal segment (Fig. 1 A); it lay in a depression formed in the skin of the flea larva which was dead. The empty egg shell (Fig. 1 A, *e*) of the parasite remained fixed to the ventral side of the sixth abdominal segment. A second cocoon contained a larger parasite fixed to the fourth abdominal segment of a resting flea larva by its head end only, the rest of the body being stretched out away from the flea larva. The flea larva was living though immobile. The

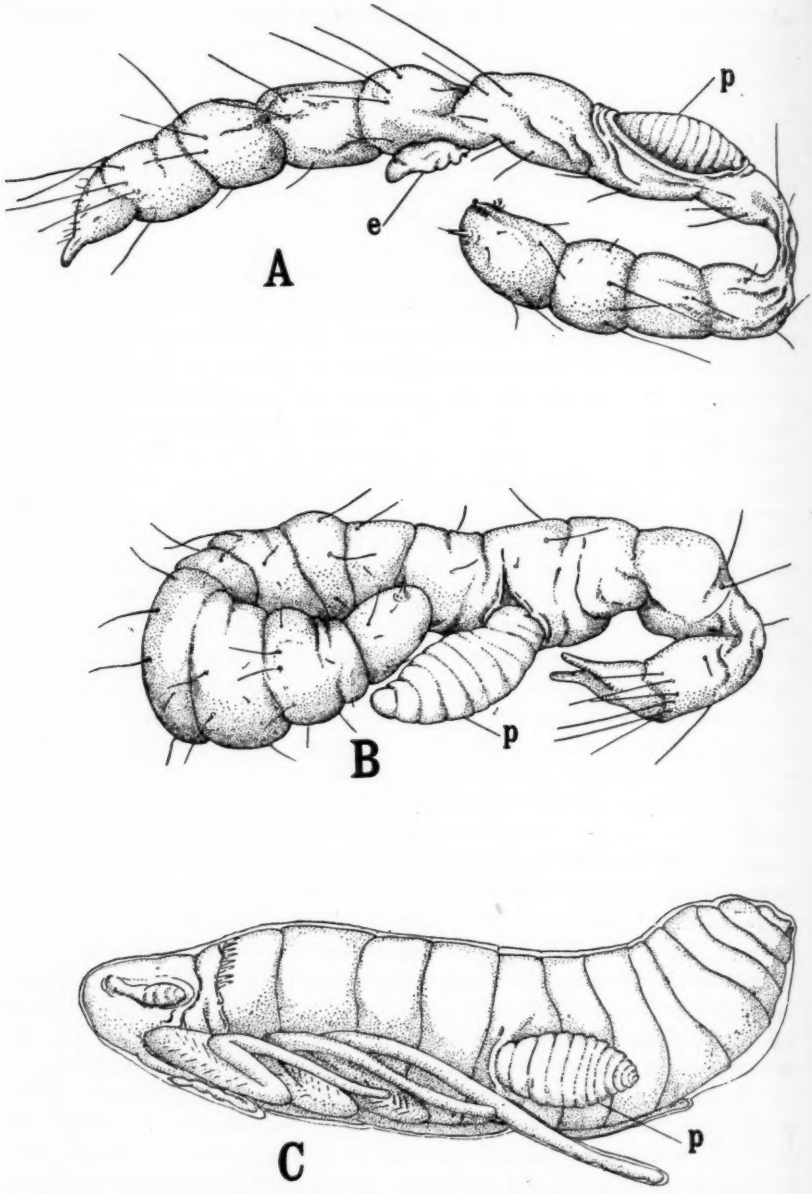

Fig. 1. A. First instar larva (*p*) and empty egg shell (*e*) of *Bairamlia fuscipes* on larva of *Ceratophyllus wickhami*, taken from the cocoon of the latter. B. Second instar larva (*p*) of *B. fuscipes* on larva of *C. wickhami*, taken from cocoon. C. First instar larva (*p*) of *B. fuscipes* on pupa of *C. wickhami*, taken from cocoon.

egg shell of the parasite was loose in the cocoon. The third parasite was attached to the lateral abdominal wall of a flea pupa, which was still alive (Fig. 1 C). The egg shell could not be found.

The second and third of these three parasites were put, together with their hosts and as much of the cocoon as possible, after dissection, into small glass tubes plugged with cotton wool (Balfour-Browne, 1922) and kept at the same temperature and humidity as before. The parasite on the flea larva moulted next day (Fig. 1 B). Both parasites, however, wandered off their hosts, and died after two days, death probably being due to exposure to air at this early stage (Haviland, 1922).

The parasite has not been reared in captivity beyond the second instar larva, though fully fed larvae have successfully reached the adult stage under experimental conditions.

It seems probable that the parasites winter as fully fed larvae. Full grown larvae obtained in October remained as larvae for more than two months when kept in the cold outdoor temperature. When some were brought into a warmer and moist place at the end of December they pupated and died as pupae or emerged and died before developing completely, as though they had been forced to pupate earlier than is normal. Attempts were made to parasitise larvae of *Xenopsylla astia* with *Bairamlia fuscipes* but without success. Larvae of *X. astia* were sent by Dr L. F. Hirst from Colombo in tubes containing dried blood and bran. Dr Hirst had put adult fleas in the tubes; these had laid eggs and died. On arrival in England the flea larvae had hatched and reached the final instar, but they did not pupate for some time. Adult *Bairamlia* parasites were put with these larvae, but died before the flea larvae began to spin cocoons.

III. EGG.

The egg of *B. fuscipes* is pure white, more or less cylindrical, rounded at the anterior end and pointed posteriorly. The chorion is covered with minute globular tubercles. The length of the egg is 0·30 mm.

IV. EXTERNAL ANATOMY OF THE LARVA.

Owing to scarcity of material it was only possible to examine the full grown larva in detail. Judging from two first instar larvae and one second instar, the external anatomy seems to resemble that of the final instar, though it was not possible to see the circumoral sense organs in these earlier specimens.

The first instar larva is 0·35 mm. long and 0·19 mm. across its widest part. The second instar larva is 0·55 mm. long by 0·23 mm. wide. There may be one or more instars as yet unknown, after the second and before the last.

The full grown larva of *B. fuscipes* is white and opaque (Fig. 2). It is composed of a head and thirteen segments. The thoracic region is slightly enlarged and the body narrows towards the posterior end. The integument is

covered with minute elevations and pits. The larva is 1·16 mm. long and 0·60 mm. wide at the second thoracic segment. The segmentation is distinct. The body is only moderately chitinised and the chitin of the head is only slightly thicker.

The head is more than twice as wide as it is long. It bears a pair of small anterio-dorsal antennae (Fig. 2 A, *a*) which are truncated and the same colour as the head. Each antenna is situated on a broad mound one-third the width of the head.

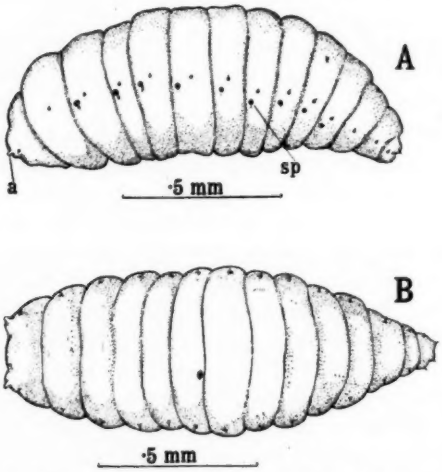

Fig. 2. *Bairamlia fuscipes*. Full grown larva. A. Lateral view. B. Dorsal view. *sp*, spiracle; *a*, antenna.

There are six pairs of sensory setae on the head (Fig. 3 A, *s.s*), anterior to the mouth. Each seta is situated on a raised circular base. The mouth is anterior and slightly ventral. There is a somewhat depressed area of thicker chitin in front of the mouth, bounded by the clypeal region of the facial arc (Fig. 3 A, *cl.f*). It bears a pair of setae on its anterior border. Dorsal to this is an area of soft chitin (Fig. 3 A, *s.a*) distinctly cut off from the rest of the head and with two pairs of setae. The labrum (Fig. 3 B, *lr*) has four pairs of sense organs arranged in two transverse rows along its border, lying immediately above the mandible. These sense organs are small circular pits, which possibly contain a minute hair in the centre.

Behind the mouth the integument is considerably smoother, and has eight pairs of circular sense organs (Fig. 3 B, *s.pt*), slightly larger than those on the labrum.

The mandibles are brown, very slightly curved in a dorso-ventral plane, and pointed. Each mandible measures 45 μ in length and articulates dorsally and ventrally with two processes of the tentorium (Fig. 4).

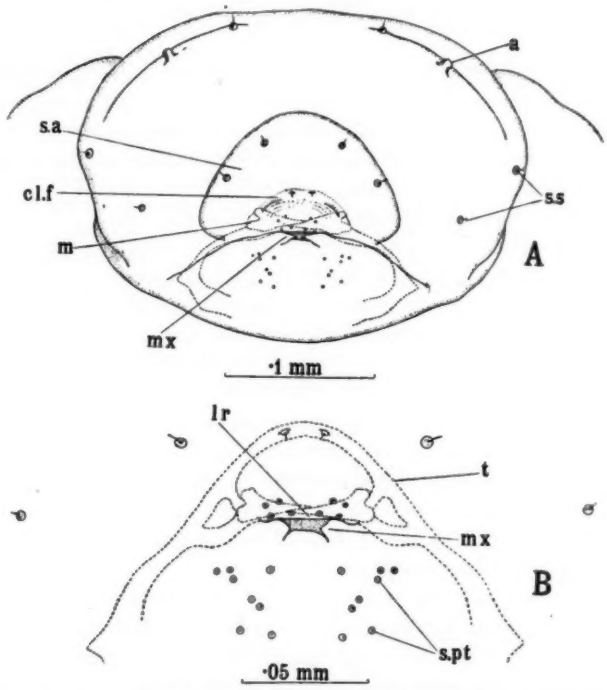

Fig. 3. *Bairamlia fuscipes*. A. Head of full grown larva, anterior view. *a*, antennae; *cl.f*, clypeal region of facial arc; *m*, mandibles seen through labrum; *mx*, maxilla; *s.a*, soft area; *s.s*, sensory setae. B. Sense pits and setae round mouth of larva (middle part of A enlarged further). *lr*, labrum with eight sense pits; *t*, tentorium seen under the integument; *s.pt*, sense pits.

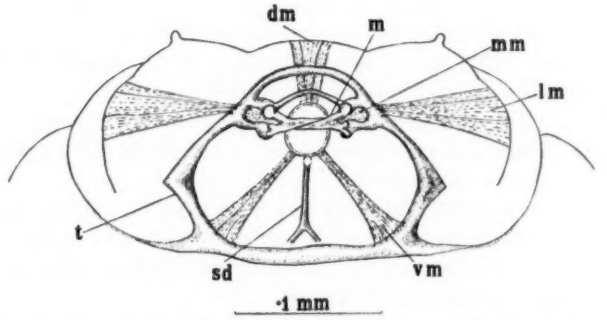

Fig. 4. *Bairamlia fuscipes*. Tentorium and muscles in the head of full grown larva. *dm*, dorsal pharyngeal muscle; *lm*, lateral muscle; *m*, mandible; *mm*, mandibular muscle; *sd*, salivary duct; *t*, tentorium; *vm*, ventral pharyngeal muscle.

On each side of the mouth there is a small triangular flap which may represent the maxilla (Fig. 3 A and B, *mx*) as in certain larvae of the Braconidae (Haviland, 1922).

The tentorium (Fig. 4, *t*) extends into the first thoracic segment and gives off lateral struts which are connected to the thickened chitinous border at the junction of the head and thorax. There are two anterior transverse bars in front of the mandibles, the ventral one being a thickening inwards of the clypeal region of the head, and continuous with the lateral arms of the tentorium, the other lying dorsally in the lumen of the head (Fig. 4).

Four sets of muscles are visible in the head. A dorsal pharyngeal (Fig. 4, *dm*) from the pharynx to the dorsal wall of the head; a pair of ventral pharyngeal (Fig. 4, *vm*) muscles from the pharynx on either side of the salivary duct, to the points where the tentorium meets the thorax; a pair of mandibular muscles (Fig. 4, *mm*) between the proximal ends of the mandibles and the tentorium; and continuing in a line with these, a pair of muscles (Fig. 4, *lm*) which extend on each side from the tentorium to the lateral wall of the head, and serve to keep the tentorium rigid against the pull of the mandibular muscles.

The salivary duct (Fig. 4, *sd*) can be seen opening into the floor of the pharynx. The duct divides before it reaches the thorax.

There is a single pair of lateral setae on each segment (Fig. 2) of the body, except the last which has two pairs.

There are nine pairs of spiracles (Fig. 2, *sp*). They occur on the meso- and meta-thoracic segments and on the first seven abdominal segments.

In several respects the larva and egg of *Bairamlia fuscipes* resemble those of the Pteromalid, *Dibrachys boucheanus* (Parker, 1924).

V. Summary.

The Chalcidoid wasp, *Bairamlia fuscipes*, was found parasitising the flea *Ceratophyllus wickhami* in the nest of the North American Grey Squirrel (*Sciurus carolinensis*) from Gerrard's Cross.

The larval and pupal stages of *B. fuscipes* were found in the cocoons of *C. wickhami*, and the adults were crawling about in the squirrel's nest.

The parasites were scarce, only 7 per cent. of the cocoons dissected at intervals during fifteen months were parasitised.

The parasite has not been reared in the laboratory beyond the second instar larva, though full grown larvae have successfully reached maturity under experimental conditions.

B. fuscipes oviposits through the flea cocoon and fixes the egg on the abdomen of a resting larva or pupa. Only one egg has been found in each parasitised cocoon. The larva feeds externally on the flea, fixing itself to the fourth abdominal segment or thereabouts.

Attempts were made to parasitise larvae of *Xenopsylla astia*, but without success.

The external anatomy of the full grown larva of *B. fuscipes* is described.

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STUDIES ON SOME SPOROZOA IN POLYCHAETE WORMS.

I. GREGARINES OF THE GENUS *SELENIDIUM*.

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(With Plates XXXV–XXXVIII and 3 Text-figures.)

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I. INTRODUCTION.

In the course of examining the contents of the intestines of polychaetes at Plymouth, I became interested in the Sporozoa that occur so frequently in these worms. When I consulted works purporting to deal with these parasites, however, I found it very difficult to get exact and reliable information concerning even the commonest forms.

I soon came to the conclusion that this unsatisfactory state of things was due in part to confusion between the different Sporozoa that may together parasitise one species of worm, and I decided to make some attempt at working out as fully as I could the life history of some very common species, already known to protozoologists, in order to discover for myself what was the real state of affairs.

I was perhaps unfortunate in my first selection. I settled down to study the parasites in *Scolelepis fuliginosa* Clpde, a worm which was easily obtainable and always richly infected: but I soon found that it harboured no less than seven protozoan parasites (five sporozoans, an astomatous ciliate and a parasitic dinoflagellate). To unravel the various life histories from one another was no easy task, but I found it extraordinarily interesting, and I got substantial support for the conclusions at which I arrived from study of comparable species in other worms.

I propose to deal in this paper with what I can contribute to our knowledge of the genus *Selenidium*. A description of an interesting coccidian from *Scolelepis*, stages in the life cycle of which have previously been confused with the accompanying "schizogregarines," will form the subject of a second paper. Elsewhere I shall deal with a cephaline gregarine and some obscure coelomic parasites, and shall also give some of my observations on the hyperparasites of certain of the larger Sporozoa.

II. THE GENUS *SELENIDIUM* GIARD.

The schizogregarines in the intestine of annelid worms have from time to time attracted the attention of protozoologists. Much interest is attached to schizogregarines because of the intercalation in the life cycle of an asexual method of reproduction, which suggests that they may form a connecting link between the coccidia and the gregarines.

Giard founded the genus *Selenidium* in 1884. "Ce sont des êtres monocellulaires à cuticle épaisse doublée par des fibres musculoïdes longitudinales et annulaires. L'espèce prise pour type, le *Selenidium pendula*, habite la cavité général" [according to Caullery and Mesnil (1899) 'en réalité le tube digestive'] "du corps des Annélides du genre Nerine. Le nom rappelle les mouvements pendulaires caractéristiques de tout le groupe." This vague definition remained until Caullery and Mesnil (1901, 1905), Brasil (1907) and Brasil and Fantham (1907) described an intracellular schizogony in the life-cycle of four species of *Selenidium*. The only species in which any details of the sporogony is known is *S. echinatum* Caullery and Mesnil (1899) from the intestine of *Dodecaceria concharum* Oerst. I cannot say how far Fantham was justified then in uniting in one diagram the schizogony and sporogony of different species of *Selenidium* from two different polychaetes (Fantham, 1908, p. 377, Fig. 3).

Brasil (1907) then further defined *Selenidium* as a schizogregarine with intracellular schizogony, having longitudinal myonemes along the whole length of the vermiform body of the trophozoite, and he proposed that Selenidiidae be regarded as a distinct family of the order Schizogregarinida, Léger (1892).

This scheme was followed by Minchin (1912), Doflein[1] (1916) and Calkins (1926)[2]; but Keilin (1923), writing of *Lipotropha macrospora* and *L. microspora*, says: "if we are to follow the tradition now established for the sub-order Schizogregarinida, we should have to introduce a new family of Lipotrophidae, since for every new genus described in this sub-order a new family has been created. This appears, however, to be completely superfluous, as it does not help to bring into this group any natural order." Wenyon (1926), following Keilin, has done away with the family name Selenidiidae.

Even a cursory study of the scattered literature on *Selenidium* shows that there is really very little exact knowledge of the life histories of the organisms grouped under this generic name. Stress is laid on the fact that there is an intracellular schizogony, but very few forms are *known* to satisfy this condition.

So far as I can discover, among the many species described, there are only four for which intracellular schizogony has been recorded[3]. These are (1) *Selenidium* sp. Caullery and Mesnil (1901) from *Scolelepis fuliginosa*, (2) *Selenidium* sp. Caullery and Mesnil (1905) from *Salmacina dysteri*, (3) *S. caulleryi* Brasil (1907) from *Protula tubularia* and (4) *Selenidium* sp. Brasil and Fantham (1907) from *Phascolosoma*. As to the facts concerning the schizogony in the first species I have great doubts, and the reasons for my scepticism are given below. Regarding the second species, I regret I have been unable to procure the host; as for the fourth, I would like to reserve my opinion until a future date, as at present I am carrying on observations on it.

Being struck by the large number of species described and the lack of evidence that they really conform to the required definition of the genus, I have taken upon myself the task of re-investigating the life cycle of the Selenidiids in some polychaetes. I do not claim to have cleared up all the discrepancies, far from it; but I think it worth while to record my observations and the interpretations to which I have been led by the facts.

III. MATERIAL AND METHODS.

The polychaete worms[4] I have investigated came from Plymouth. On various occasions I myself went down to Plymouth and made the collection. At other times I had the living specimens sent up to me in London.

Myxicola infundibulum Mont., *Branchiomma vesiculosum* Mont. and *Sabella pavonina* Sav. came from Salstone (Salcombe), where they are found in abundance between the tide-marks; *Pomatoceros triqueter* L., *Cirratulus cirratus* Mull., *Scolelepis fuliginosa* Clpde and *Terebella lepidoria* Kühl. came from Rum Bay. Large numbers of worms of each species occur together, and this

[1] Doflein and Calkins spell the family name Seleniidae.

[2] The date quoted by Calkins for founding the family "Seleniidae" is 1905, but he is apparently wrong, for Brasil's observation on the schizogony of *S. caulleryi* was not published till 1907.

[3] Caullery and Mesnil (1899) also give a figure of "endogenous multiplication" in one of the species of *Selenidium* from *Cirratulus cirratus*, but they put a mark of interrogation against it.

[4] My thanks are due to Mr D. P. Wilson, Assistant Naturalist, Marine Biological Association, Plymouth, for kindly identifying the worms for me.

no doubt accounts for the high percentage of infection in worms living in the contaminated medium; but each species of worm has its own peculiar gregarines. Specimens of *Scolelepis fuliginosa* were observed monthly throughout a whole year; the other polychaetes were examined more casually, at intervals of about 3 months. In London I kept them in aerated vessels with frequent change of sea-water. In this condition they appear to remain healthy for about 4 to 6 weeks, after which they become very inactive and ultimately die.

The large polychaetes such as *Myxicola*, *Branchiomma* and *Sabella* were narcotised in weak alcohol (10 per cent. in sea-water) before their gut contents were examined. This preliminary narcotisation is essential because the violent contraction of the living muscle renders it very difficult to dissect out the alimentary canal for examination. Small portions of the alimentary canal from along its whole length were teased separately on slides and searched for the protozoan parasites. The small size of *Scolelepis* and *Pomatoceros* makes it extremely difficult to get out the alimentary canal entire; hence it was found best to cut the worms transversely into small pieces, which were separately embedded and sectioned, after a drop of the gut contents had been examined. The gut of the smaller specimens of *Scolelepis* can be directly examined under the microscope by applying a gentle pressure on the cover-glass; this stops their wriggling movement and at the same time makes them more transparent.

The parasites were always examined in the fresh condition and, as far as possible, in their natural fluid media. When necessary, the natural fluid was supplemented with sea-water. The sea-water does not seem to have any immediate deteriorating effect on these parasites, as some of them did not show any change of form when kept in it for 6 to 12 hours (or sometimes even longer). The associating gregarines, as shown later (see p. 384) appear to be still more resistant than the trophozoites.

Neutral red (0·1 in 4000) was generally used as intravitam stain. 1 per cent. methyl green in 1 per cent. acetic acid in water was found very useful for staining the myonemes, though it kills the organisms quickly.

Fixed and stained preparations, smears as well as serial sections, were made for detailed study of the morphology and the elucidation of the life history of the parasite. Smears were fixed in Schaudinn's fluid and Bouin and a modification of Bouin's fluid[1]. I got the best result with the last named fixative. Fifteen minutes to 1 hour's fixation was found to be quite satisfactory for the smears. The same fixatives, as well as Flemming's fluid (both weak and strong formulae) and Zenker's fluid, were used for the material to be embedded; and here again I found modified Bouin's fluid by far the most satisfactory. The gut or the worm to be fixed was cut into small pieces, and kept in the fixative for 12 to 20 hours. The pieces of *Scolelepis* go into all sorts of curves on the addition of

[1] Sat. picric acid in 90 % alcohol ... 2 parts
Sat. $HgCl_2$ in dist. water ... ... 3 parts
Formalin ... ... 1 part
Gl. acetic acid ... ... 2 parts

the fixative, but this was overcome by quickly pressing them back to their normal position by means of a soft camel-hair brush. 70 per cent. alcohol containing a small quantity of lithium carbonate was used to wash out the picric acid from the material fixed in this fluid; immersion of smears for 30 minutes to 1 hour and of the tissues for 2 to 6 hours in this medium removes from them the visible traces of picric acid without causing any apparent injurious effect.

The stains used were as follows: Mayer's acid haemalum (15 minutes to 1 hour), Ehrlich's haematoxylin (10 to 30 minutes), Delafield's haematoxylin (2 hours to overnight), borax carmine (15 minutes to 1 hour), picro-carmine (10 to 30 minutes), Dobell's haematein (15 minutes in the mordant and 10 to 15 minutes in the stain, followed by differentiation in acid alcohol), Dobell's modification of Mann's methyl-blue-eosin, and Heidenhain's iron haematoxylin (15 minutes to 1 hour in the mordant and about the same time in 1 per cent. ripened haematoxylin in distilled water). The times mentioned here refer both to smears and sections. Sometimes the haematoxylin preparations, especially the Heidenhain ones, were counterstained with eosin, orange G or light green.

The observations recorded here are mostly based upon the material fixed in Bouin-Duboscq's fluid, and stained either in Heidenhain's haematoxylin or in Dobell's modification of Mann's methyl-blue-eosin.

Sections from 6 μ to 8 μ thick were found most useful for the study of the parasites in the gut wall of the worms.

IV. *SELENIDIUM CAULLERYI* BRASIL.

When I decided to deal with *Selenidium* I thought it advisable to work first at *S. caulleryi*, the species described by Brasil from *Protula tubularia* Mont., on which, so far as I know, no further report has been made since 1907. I chose this species since Brasil's account of the schizogony is given in some detail. Unfortunately I had great difficulty in procuring this worm, but in one from Plymouth which was heavily infected, I have the stages described by Brasil[1]. Two of these are shown in Text-figs. 1 and 2. The parasites were especially abundant in the hinder part of the gut. I have been able to follow the escape of the full-grown trophozoites into the gut, where they occur in the intestinal crypts in numbers. I am able to amplify an observation of Brasil's which is of importance in enabling one to trace the organisms with some certainty through the various stages of the life history. This is the presence at the anterior end of the gregarine of six to eight refringent intracytoplasmic thread-like bodies ("lignes sidérophiles" of Brasil), which, when full grown, are about 4 μ long: they seem to be homologous with similar structures which I shall describe below in other species of *Selenidium*. They

[1] From so small a material it would be rash to conclude that *Protula* harbours no other Sporozoa than the *Selenidium*. But I see no reason to doubt that all the stages belong to the organism, and the general similarity of the process here to that in the *Selenidium* from *Myxicola*, of which worm I had abundant specimens, confirms my opinion.

stain black in iron haematoxylin and red in Mann's methyl-blue-eosin. I find that these threads appear in the more darkly stained anterior ends of very young trophozoites about 6–8 μ long and 4–6 μ broad, while these are still intracellular and before any epimerite has been developed. (Brasil found the "lignes sidérophiles" only when the preparation had been insufficiently differentiated and apparently not before the trophozoites had reached a length of 40 μ.) The trophozoites, when about 15–20 μ long and 8–10 μ broad, slip out of the host cell, hind-end first, into the lumen of the gut, retaining their hold

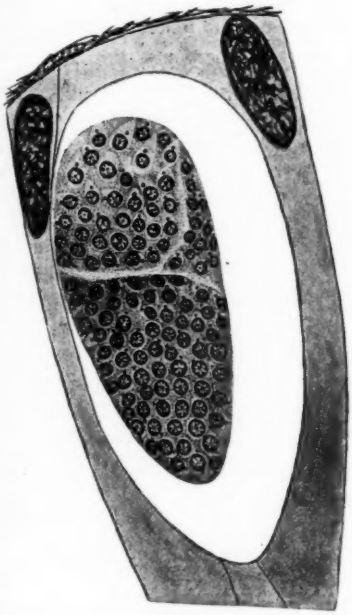

Fig. 1.

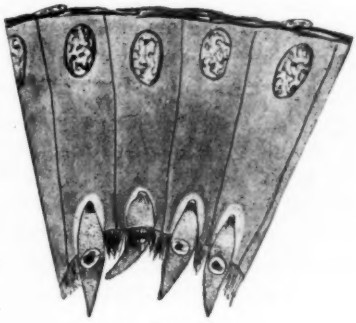

Fig. 2.

Text-fig. 1. *Selenidium caulleryi* Brasil. Intracellular schizont dividing up into a large number of merozoites. Drawn from a section of the intestine of *Protula tubularia*. (× 1625.)

Text-fig. 2. *Selenidium caulleryi* Brasil. Young individuals entering the epithelial cells of the intestine of *Protula tubularia*. (× 1150.)

on the host cells for a time by means of the knob-like anterior end within which the dark-stained threads appear (Pl. XXXV, fig. 1) and so pass the rest of their life as extracellular parasites.

Here I would point out that Brasil (1907) on Pl. XV, fig. 5, shows the section of a multinuclear schizont, and alongside the nuclei are lying minute spherical dark-stained granules, of which he does not trace the further development and as to the functions of which he is doubtful; but from what I have seen in *S. mesnili* (see p. 379) I conclude that these granules form in *S. caulleryi* also the basis in the merozoites of the groups of dark-staining intracytoplasmic

threads to which he refers (though he shows them only in one figure) and which I find constantly present in the half-grown and full-grown stages.

Later on, as I have said, the organisms detach themselves and are free in the lumen of the gut; they have now increased considerably in size and measure 75–85 $\mu \times$ 20–25 μ. At this stage I have seen the onset of sporogony, which part of the life-cycle Brasil did not describe, though he refers to "le début de l'enkystement."

Before association, and even while the parasites are still intracellular, the nuclei of the gametocytes elongate transversely to the long axis of the body, the karyosome fragments, and there is every indication that the first spindle in the production of the gamete nuclei is being precociously prepared. After their association, which takes place by their posterior ends, the gametocytes thicken and shorten; the dark-staining threads at their anterior ends can still be seen. At a somewhat later stage, their nuclei present a very curious appearance, which I am at a loss to interpret. Within the transversely elongated nuclear area appear a large number of vesicles, each about 4 μ in diameter, which have chromatin in them, usually arranged in a crescentic patch on one side (Pl. XXXV, fig. 2). In other specimens similar bodies of the same size, which are here undoubtedly the gamete nuclei, are found distributed throughout the cytoplasm, where they show a tendency to accumulate towards what was the anterior end of each gametocyte (Pl. XXXV, fig. 3). Occasionally I have noticed, prior to the formation of the intranuclear vesicles, what look like small, rather ill-defined spindles. The inference is that the gamete nuclei are actually formed within the nuclear area of the parent gametocyte, and then wander out into the cytoplasm to take up their definitive position. But I admit the possibility of intranuclear parasitism, and I think it best merely to state what I have seen and refer the reader to the figures. I find Caullery and Mesnil (1899) picture and describe this same phenomenon where they are dealing with living *Selenidium* from *Scolelepis fuliginosa*[1].

I have not seen the gametocytes of this *Selenidium* go beyond this stage, and therefore unfortunately I can say nothing as to the form of gametes and spores. It is true that my results are drawn from a single worm, and with more material at my disposal, I might have been more fortunate; but my study of gametocytes in other species of *Selenidium* (see pp. 380, 384, 385) inclines me to think that in this condition the sexual forms leave the body of the worm and finish development either in the sea or in the body of some other animal that ingests them.

[1] "Pendant ce temps, le noyau est le siège de phénomènes que nous n'avons malheureusement pas pu étudier sur des préparations colorées, mais dont la signification ne nous paraît pas discutable. Le noyau s'allonge transversalement de manière à occuper tout un équateur du parasite; à son intérieur, on voit un assez grand nombre de granules assez réfringents qui nous paraissent d'un émiettement du karyosome. Bientôt, les parois nucléaires disparaissent et tous les karyosomes secondaires se portent à la périphérie. Ils deviennent là, sans doute, les centres de formation des noyaux des sporoblastes."

V. *SELENIDIUM MESNILI* BRASIL.

In 1909 Brasil described *S. mesnili* from the digestive tube of *Myxicola infundibulum* Mont., which he commonly found very richly infected[1]. Considering the importance he attaches to an intracellular schizogony in *Selenidium*, it is surprising to note that Brasil placed this parasite in the genus *Selenidium* without having seen its asexual reproductive phase ("je n'ai pu reconnaître chez *Selenidium mesnili* aucune indication de multiplication schizogonique" (Brasil, 1909, p. 111)). He never saw the gregarines free in the lumen of the gut, and was doubtful whether a sporozoite at the beginning passes at once into the epithelium or at first lies on the surface and penetrates gradually. He also observed and figured chromatic granules, irregularly ovoid and of variable dimensions, at the blunt anterior end of the organism. My own observations, however, make it clear that intracellular schizogony does take place and that Brasil was justified in his assumption that this gregarine from *Myxicola* is a true *Selenidium* according to his own definition.

Like Brasil, I have found that almost every *Myxicola* I examined was infected with this parasite[2] (out of the 200 worms examined, 185 were parasitised). *Myxicola* belongs to the tube-dwelling polychaetes placed in the sub-order Sabelliformia. It secretes a gelatinous tube, within which it retreats on disturbance. Though it is one of the "sedentary" worms, it can readily leave its tube and swim freely, tail foremost. This peculiar type of locomotion is perhaps responsible for the restriction of the parasites to the anterior half of the gut; so far as I am able to judge from my own preparations, they never appear in the posterior half of the digestive tube. The worms are found in mud between the tide-marks, and almost a handful can be collected from the same spot; this perhaps accounts for the rich infection. In contrast to what happens in *S. caulleryi*, the greater part of the life cycle of this parasite is passed within the host cell, and it is only the mature gametocytes which are found free in the lumen of the gut.

When examined fresh, a parasite executes the movements characteristic of the genus—generally it bends from side to side; the extreme anterior end is slightly mobile and sometimes the anterior half of the body makes a rotating movement while the tail end is kept still. Of course, these movements are only visible when a parasite is liberated from the infected host cell, within which, otherwise, it is in a more or less inert condition. I have failed to observe any movement of translation such as is shown by *Monocystis* or *Lankesteria*.

An average-sized trophozoite measures about $50–90\,\mu \times 20–30\,\mu$, but sometimes individuals measuring $190\,\mu \times 52\,\mu$ are also met with in smears and sections. The body is shaped like a spindle with the anterior portion slightly broader than the posterior. The anterior extremity ends in a dome,

[1] Roskin and Levinson (1929) have referred to *S. mesnili*, but from their sketch it is very difficult to believe that they were dealing with this species.

[2] I have seen no other protozoan parasite in the gut or coelom of *Myxicola*, and am quite confident that every stage I describe here belongs to the same life history.

the centre of which is slightly drawn out into a clear hyaline nipple. There is always a circlet of club-shaped refringent bodies, about six to eight in number, situated at the base of the nipple (Pl. XXXVI, fig. 4). Brasil describes them as consisting of a homogeneous substance and thinks they are the result of metabolic activity (see below).

There is a definite cuticle marked by grooves running parallel to the longitudinal axis of the body. In very young parasites these grooves are imperfectly visible, but as the parasite grows they become more and more pronounced; the number of these grooves seems to vary between 24 and 30. Along them run the longitudinal myonemes which give a striated appearance to the stained preparations. Just below the cuticle, granules in the cytoplasm appear to be arranged in transverse rows which are visible even in the fresh specimens. In a stained specimen they give the body the "zebroid" appearance noted by Brasil. The rest of the cytoplasm is very finely granular and in fixed specimens is alveolar.

The nucleus is usually situated in the middle of the body and is perfectly spherical. A clear space is often seen between the nuclear membrane and the main nuclear mass; the space is traversed by fine strands (Pl. XXXVI, fig. 4, *a*, *b*). The main nuclear mass is granular and stains blue with Dobell's modification of Mann's methyl-blue-eosin. It contains a large spherical karyosome[1], usually accompanied by a small chromatic spherule. Sections stained in Mann's methyl-blue-eosin and Delafield's haematoxylin show that the karyosome is not homogeneous, as might be supposed from preparations stained in iron haematoxylin, but appears as a blue-staining vesicle containing two to four pink granules. The accompanying intranuclear spherule and the club-shaped bodies at the anterior end of the organism also take up the pink stain. In a young trophozoite there is only one pink-staining granule within the karyosome and there is no intranuclear spherule.

The beginning of the nuclear division is seen in some of the intracellular trophozoites which have attained a dimension of about $190 \times 40 \mu$. Their nuclei assume an elliptical or spindle shape, and the elongation is transverse to the long axis of the body. Usually one karyosome-like body is seen lying at either pole of the spindle; sometimes such bodies are seen lying within it. I cannot say whether these bodies are derived from the original karyosome or are formed anew within the nucleus, but it does appear that the karyosome shows an increase in the amount of the pink-staining element as the trophozoite advances in age, and it is most likely that a certain amount of this is discharged at the onset of schizogony. These large schizonts are very common in transverse sections of the digestive tube. I have not come across forms with two or four nuclei, and the next stage that appears in my preparations shows advanced schizonts containing numerous small nuclei (about 200 to 300); I think it possible that there may be a multipolar fission. In these nuclei

[1] Throughout this paper I have used this term for the dark-staining spherical body in the nucleus. I prefer not to commit myself to speculation as to its true nature.

the staining substance is confined to the periphery of the nucleus (Pl. XXXV, figs. 5, 6) and there are no karyosomes. Each nucleus is accompanied by a darkly staining granule (Pl. XXXV, fig. 6), probably the precursor of the club-shaped bodies at the anterior end of the adult. These granules may or may not be the outcome of the activity of the nucleus, but the fact that they are not formed by the splitting of the original club-shaped bodies is made obvious by the persistence of these in an advanced schizont. Later on, each small nucleus is surrounded by a portion of cytoplasm, and in this way arises a group of pear-shaped merozoites. The nuclear end of a merozoite is broader than the other end and is the posterior end of the organism. As growth proceeds, the extranuclear granules within the merozoites show signs of dividing by a median constriction (Pl. XXXV, fig. 7). A merozoite in a newly invaded cell loses its pointed anterior end, assumes an oval shape, and a karyosome is now developed in its nucleus (Pl. XXXV, fig. 8). The staining substance in the nucleus is distributed towards the nuclear membrane and is connected to the karyosome by means of fine strands (Pl. XXXV, fig. 8). The extranuclear chromatic granules, which are now about six to eight in number, are irregularly scattered at what I believe to be the anterior end of the merozoite. These merozoites seem to invade the neighbouring epithelial cells directly after the group has been broken up, for no free stage similar to that in *Selenidium caulleryi* could be found in the lumen of the gut. They occur in swarms within the host cells, which, as the effect of parasitism, shrink away from the neighbouring tissue and form a sort of "peninsula" (Pl. XXXVI, fig. 10). These "peninsulas" are very common in my preparations, and the continuity of the gut wall is certainly impaired by such interruptions.

As the organism grows larger, the nucleus takes up a more or less central position and the longitudinal striations and the "zebroid" nature of the adult cytoplasm appear. The staining substance of the nucleus, which was previously distributed on the nuclear membrane, now recedes and is connected therewith only by means of a few strands (Pl. XXXV, fig. 9). Such stages when stained in Mann's methyl-blue-eosin show that the pink-staining material of the nucleus is confined to the karyosome and that the rest of the nuclear mass stains blue. The extranuclear granules are here still scattered irregularly at the anterior end and are now ten to twelve in number (Pl. XXXV, fig. 9), with an average length of 2μ. As growth proceeds, the hyaline nipple of the dome-shaped anterior end becomes differentiated; the periplast becomes well marked by the longitudinal grooves; the chromatic granules in the cytoplasm, which are now about six to eight in number and have assumed a club-shape, arrange themselves at the base of the nipple. The increase in the number of the extranuclear granules during the growth period and their ultimate reduction in number in a full-grown trophozoite is perhaps associated with metabolic changes in the parasite during the growth stages. (In some average-sized specimens I have often seen two or three granules smaller in size than others, and I presume that these are just being absorbed.)

In sections of the gut, only the associating forms are found in its lumen.

It appears that some of the trophozoites measuring 150–190 $\mu \times$ 40–50 μ, instead of carrying on the process of schizogony, drop out of their host cells and associate by their posterior ends. When examined alive the newly paired individuals show slight to and fro movement of their anterior ends, where the refringent club-shaped bodies are still visible. Gradually they shorten and a tough cyst wall is secreted round them, giving rise to a spherical gametocyst 40–50 μ in diameter. Unfortunately I can say very little about the nuclear division in a gametocyst of this species, as, for some reason or other, this is not very definite in my preparations; occasionally the nuclei are seen to have assumed a dumb-bell shape with darkly staining caps at either pole. Such stages are found in abundance within the first few segments of the digestive tube, and they are perhaps thrown out in the sea-water either when the worm suddenly contracts into its tube, as it does at the slightest disturbance, or else they drop out through the mouth when the worm swims freely, hind end foremost.

I have not yet seen spore formation in this species. I believe it normally occurs after the gametocysts have left the body of the host.

VI. *SELENIDIUM* FROM *SCOLELEPIS FULIGINOSA* CLPDE.

I propose now to give some account of two species of *Selenidium* with which the gut of almost every specimen of *Scolelepis* collected at Plymouth is infected. I have examined as many as 300 worms, and the guts of 290 of these were infected with the first species and 220 contained the second species. Both species were rather vaguely described by Caullery and Mesnil (1901) and, as far as I know, they have not been studied since. The abundance of material and the richness of infection within the individual worms inclined me to re-investigate these parasites more particularly with reference to the intracellular schizogonic phase recorded by Caullery and Mesnil. The description by these authors of what they observed does not agree with comparable stages in *S. caulleryi* Brasil from Protula and *S. mesnili* Brasil from *Myxicola*.

I have cut serial sections of 300 worms and have studied my sections with the utmost care, but though the gregarines were found in 97 per cent. of the specimens in one case and in 70 per cent. in the other, up till now I have failed to find *Selenidium* of either species undergoing schizogony, whether intracellular or extracellular. Certain peculiar appearances are produced by parasitic organisms within the cytoplasm of these gregarines when free in the gut, and at first I thought I had to do with the onset of extracellular schizogony; the occasional presence of a dividing parasitic dinoflagellate of similar dimensions to *Selenidium* also misled me for a time. The intracellular schizogony, referred to by Caullery and Mesnil and quoted by subsequent writers as further support for the given definition of the genus, certainly occurs in abundance, but, in my opinion, it has nothing whatever to do with *Selenidium*: as will be seen from the description I shall give in a later paper, it is a part of the life cycle of an accompanying but totally unrelated sporozoan, the sporogony of which I have also been fortunate enough to observe.

(*a*) MORPHOLOGY OF THE TROPHOZOITES.

Selenidium spionis (Köll.) (= *Gregarina spionis* Köll. = *Polyrabdina spionis* Ming. (pro parte) = *Selenidium* sp. No. 1 Caullery and Mesnil).[1]

About 97 per cent. of the worms from Rum Bay, Plymouth are infected with this organism. The gut along the first 25 to 30 segments is lined with a thicker epithelium than occurs further back and it appears to be free from any parasitic invasion.

The living trophozoite executes the movement characteristic of the genus. When detached it bends rhythmically from side to side, bringing anterior and posterior ends together and then straightening the body again. When attached to the epithelium of its host by its anterior end it has a pendular movement; I have never observed any movement of translation.

Individuals with dimensions varying from 30 to 300 μ in length and 25 to 50 μ in breadth are to be found both in smears and sections of the gut. There is a definite cuticle, which is marked by 24–30 longitudinal striations. The anterior end, which is broader than the posterior, has a spoon-shaped depression on one side and a number of darkly staining thread-like structures about 12 μ long are situated just below the cuticle. In the fixed preparations and also in the fresh material, the more hyaline portion of the endoplasm can always be distinguished between the transverse denser areas. Both in the fresh specimens and in fixed preparations a peculiar streak can be seen lying along the whole of the median length of the body, gradually fading away towards either extremity. The body is elliptical in transverse section, and half-way between the anterior and posterior ends this streak expands and includes the nucleus within it; a definite membrane can also be seen round the nucleus (Pl. XXXVII, fig. 18). I think this area is associated with the bending movement of this *Selenidium*, and I suggest that it is a median canal with which there is a cytoplasmic connection to all the transverse dark areas or the fibrils, and that, when the organism bends towards one side, the cytoplasm from that side flows into the median canal and is perhaps then drawn into the fibrils of the convex side on which there is now a great pressure. In transverse sections from both pre- and post-nuclear regions one can often see fibres which are connected to the longitudinal canal and run towards the edges (Pl. XXXVII, fig. 19).

The nucleus is oval and is situated in the middle of the body: its long axis is parallel with that of the body. It contains a spherical karyosome which is

[1] Kölliker (1845 and 1849) described *Gregarina spionis* from the gut of an undetermined species of *Spio* (=*Scolelepis*) at Naples. Mingazzini (1891 and 1 93) described a "dimorphic" gregarine from *Spio fuliginosa*, which he called *Polyrabdina spionis*. Caullery and Mesnil (1914) were undoubtedly right when they pointed out that Mingazzini's "*Polyrabdina*" really included two distinct kinds of gregarine, and they reserved the name of *Polyrabdina spionis* for the dicystid. It is clear from Mingazzini's figures that his second form was a species of *Selenidium* and it bears most resemblance to Caullery and Mesnil's *Selenidium* sp. No. 1 (1899); this also agrees very well with Kölliker's original description of *Gregarina spionis*.

usually placed slightly towards one pole. Here again the karyosome and the threads of the anterior extremity of the gregarine take up the pink stain when stained with Mann's methyl-blue-eosin, the rest of the nucleus staining blue. With iron haematoxylin the karyosome and the threads stain black.

I have not come across any intracellular stages, and the great range of variation in the dimensions of the parasites suggests that most of the growth takes place outside any host cell. Very occasionally I have found a few full grown individuals lying embedded in the gut wall under the epithelial layer and parallel with the longitudinal axis of the body of the host (Pl. XXXVIII, fig. 33). These were not actually within cells, and I consider their imprisonment in the tissue to be an accident. As a result of parasitism, the nucleus of the cell to which the trophozoite is attached is very much hypertrophied. This fact has already been pointed out by Caullery and Mesnil.

Selenidium foliatum n.sp. (= *Selenidium* sp. No. 2 Caullery and Mesnil).

About 70 per cent. of the *Scolelepis* from Plymouth were found to be infected with this second species of *Selenidium*. It appears side by side with *S. spionis*, but can readily be distinguished from this by its flattened leaf-like shape and by its comparatively narrow and elongated nucleus. In the fresh condition it displays characteristic rhythmical snake-like movements; the body is thrown into a **S**-shape or is twisted spirally. When attached to the epithelium of the host by its anterior end, it shows a pendular movement. No movement of translation has been observed.

The dimensions vary between 30 and 250 μ in length and 10 and 30 μ in breadth. The anterior extremity is broader than the posterior and has a cone-shaped depression running into the endoplasm; the wall of this depression is lined by a number of refringent thread-like structures about 8 μ long, which, together with the circular edge of the cone, stain deeply with iron haematoxylin (Pl. XXXVI, fig. 13). Along the median line of one of the flattened faces, the body is raised into a prominent ridge (Pl. XXXVI, fig. 13 *a*) which gradually fades away towards the posterior end; this gives the transverse sections the **T**-shaped appearance referred to by Caullery and Mesnil. The cuticle is not so thick as in the previous species, and consequently the longitudinal striations, 16 to 24 in number, are also more faintly marked. The endoplasm is finely granular.

The nucleus is situated in the middle of the body and is elongated antero-posteriorly; there is a thin nuclear membrane, and a space between it and the main nuclear mass can always be distinguished. The spherical karyosome lies usually towards the anterior end of the nuclear mass, which is dotted with darkly staining granules. The shape of the nucleus, the peculiar disposition of the karyosome and the invariable occurrence of the thread-like structures at the anterior end of the parasite are the salient features which help one to distinguish the young intracellular stages (Pl. XXXVI, fig. 11) of this organism from the similar stages of the coccidian that may occur along with it. Young intracellular stages have their anterior ends directed towards the base of the

epithelium. It appears that a sporozoite, after entering the host cell, grows there for a short time, and then, while only 24–30 μ in length and 6–8 μ in breadth, gradually pushes its way out backwards, remaining attached to the cell by its anterior end till the gametocyte stage is reached (Pl. XXXVI, fig. 12).

(*b*) SPOROGONY.

Sporogony in *S. spionis* and *S. foliatum* is described here for the first time. This is of the greater interest, since the only other description of spores in *Selenidium* (*S. echinatum* Caullery and Mesnil) from *Dodecaceria concharum* is very inadequate.

It was suggested to me by Mr Dobell that the scarcity of stages beyond association in the lumen of the gut might perhaps mean that further development in the associates took place outside the host. Consequently I searched through the mud in which *Scolelepis* lives normally. I had almost given it up in despair, when accidentally I came across some spore-containing gametocytes among the débris around worms kept in sea-water in the laboratory. Since then the following observation was made about half a dozen times, and every time it was a success. Six to a dozen *Scolelepis*, after being scrupulously cleansed of grit and mud, were kept in sea-water under aeration; and within 48 to 60 hours spore-containing gametocysts could be picked up under the microscope from among the débris which is mixed with the eggs, etc., adherent to the slimy secretion from the skin of the worm. This accounts for the absence in the lumen of the gut of stages later than association, and suggests that association forms, at a certain stage, need to come out into the sea-water in order to form spores. These forms might either be thrown out by the peristaltic movement of the gut or by the autotomy which occurs very frequently. On two occasions the formation of the gamete nuclei has been observed while the gametocytes were in the lumen of the gut, and this, therefore, does not completely eliminate the possibility of the spores being occasionally formed there.

Two types of gametocyst are found in the débris—an oval type, 102–108 μ long, and 60–65 μ broad, and a spherical type, 65–70 μ in diameter; and I can show that the former belongs to *S. spionis* and the latter to *S. foliatum*.

Smears or sections of spores or gametocysts fixed in modified Bouin's fluid or Schaudinn's fluid and stained in iron haematoxylin were of very little use in determining the number of nuclei in the sporoblasts; with Delafield's haematoxylin only a slight degree of improvement was obtained. Better results were only obtained when the gametocysts were fixed in Dobell's modification of alcoholic Bouin (a few drops of chloroform to be added to the fixative just before use) for 24 hours (1 hour on the top of the bath and 23 hours at room temperature) and stained in iron haematoxylin. Sections 4 μ thick were found to give satisfactory results. Careful camera lucida drawings of spores showing the number of sporozoites were also made from the fresh material. I shall now describe the process, first in *S. spionis* and then in *S. foliatum*.

Spore formation in Selenidium spionis.

Prior to association certain changes take place in the gametocytes lying within the lumen of the gut. The median longitudinal canal disappears, the pointed posterior end becomes flattened and spatulate, and the characteristic bowing movement is less frequent than in the ordinary trophozoite stage. Within the nucleus appear numerous small vesicles, just as in *S. caulleryi* and in other species (see pp. 376, 385, 390). The thread-like bodies at the anterior end of the gregarines are still visible. Gametocytes in this condition are fairly frequent in the débris around the worms kept under aeration, and when examined under the microscope present quite a healthy and normal appearance.

Text-fig. 3. Gametocyst of *S. spionis* (Köll.). Microphotograph taken from a section of *Scolelepis fuliginosa*. (×1000.)

The pairing gametocytes are approximately equal in size, 250–300 μ long and 50–60 μ broad; they associate by their posterior ends. This process agrees with what I have observed in other species of *Selenidium*, but is quite unlike that in *Selenidium echinatum* Caullery and Mesnil (1899) from *Dodecaceria concharum*, where the gametocytes are said to associate by the ends corresponding to their epimerites. The nuclear membrane persists for a time even after the gametocytes have associated. Gamete nuclei seemingly identical with the intranuclear vesicles of an earlier stage are now scattered all through the cytoplasm (Text-fig. 3). The anterior dark-staining threads have disappeared, but the characteristic striations on the body still persist and can be counted.

Both the associates are active for some time, but gradually they shorten and become reduced in size, until finally a common cyst wall is formed enclosing the two, and thus giving rise to an *oval* gametocyst, 102–108 μ long, 60–65 μ broad. Further stages in gamete formation and the actual process of fusion of the gametes have unfortunately escaped my observation. It would appear that further development does not normally occur within the host, and the gametocytes are now passed out into the water. The next stage I have found shows numerous (about 80 to 100) fully formed sporoblasts. No residual mass is left in the centre of the gametocyst. Each oval sporoblast, 20–26 μ long and 12–14 μ broad, is then surrounded by a smooth sporocyst. The sporoblast gives rise to four elongated sporozoites, pointed at both ends, each about 20–22 μ long and 2–4 μ broad[1]. A refringent residual mass of protoplasm is left in the centre of the group and shows clearly in the fresh condition but is readily dissolved in the fixative. The nucleus of the sporozoite is central in position and a refractile area can be distinguished at one end, which presumably is the precursor of the anterior thread-like bodies of the adult (Pl. XXXVII, fig. 21). Usually at this stage the wall of the gametocyst bursts and the spores are liberated. After a gametocyst has reached the sea-water it takes about 48 to 60 hours to develop the spores at 15–18° C.

Spore formation in Selenidium foliatum.

The gametocytes either detach themselves from the host cell prior to association or associate *in situ* by their posterior ends. The pairs of the latter kind are much smaller in dimensions than the former and suggest that the association is precocious. In either case the two associates are often of very different sizes. In the débris of worms kept under aeration I have often come across pairs in which the larger is 230–250 μ long and 15–30 μ broad and the smaller 40–50 μ long and 15–30 μ broad. When the associates are stained *intra vitam* with neutral red, the cytoplasm of the shorter of the two behaves differently from that of the longer and shows innumerable pink granules. The ventral ridge persists for some time and then begins to fade as the gametocyte advances in age. The same fate overtakes the thread-like structures at the anterior extremity. Owing to the considerable difference in bulk and to the persistence of morphological features such as the characteristic striations, it is quite easy to distinguish the gametocysts of this species from those of *S. spionis*. The nuclei of the gametocytes behave in the same peculiar way as in the previous species. Unfortunately I have not seen gamete formation or zygote formation, and the next stage that I have found is the advanced gametocyst containing sporoblasts. This I have never found within the body of the worm. A gametocyst of *S. foliatum* can be readily distinguished from that of *S. spionis* (along with which it is commonly found) by its much smaller size—68–70 μ in diameter—

[1] *S. echinatum* is the only species in which sporozoite formation has been described hitherto (by Caullery and Mesnil, 1899); there also the number is stated to be four, but the spore wall is roughened by spines.

as is natural, when one compares the bulk of the associates in the two species. Moreover it is perfectly spherical and contains only about 40 to 50 sporoblasts.

Each oval sporoblast is surrounded by a tough smooth sporocyst. The finished spores are 20–22 μ long and 18–20 μ broad and contain eight spindle-shaped sporozoites, arranged in a bundle, with a mass of refringent residual protoplasm lying in the centre. Each sporozoite is 20–22 μ long and 4–6 μ broad, has a central nucleus and a refractile area at what I take to be its anterior extremity[1] (Pl. XXXVI, fig. 15). At this stage the gametocyst bursts, and the liberated spores lie among the débris ready for ingestion by another worm. It takes about the same time as in *S. spionis* to form spores from gametocysts under similar conditions.

(*c*) Method of infection.

The smallest trophozoites found in the gut of *Scolelepis* are not much larger than the sporozoites. I have described intracellular stages of *S. foliatum* which are only 24 $\mu \times 6\,\mu$ in size, and it seems reasonable to suppose that the spores are swallowed by feeding worms, hatch in the gut and liberate the sporozoites, which then burrow their way into the epithelium or directly attach themselves thereto. If it could be shown that this actually happens, then the possibility is ruled out that some stage in the life history, such as the missing schizogony in these species, takes place in another host. Accordingly, I fed worms on spores. To do this, I first of all procured some mature spores as described above (see p. 383), and then took several lots of six to a dozen *Scolelepis* and kept them in sea-water which was inoculated with spores. At intervals of 24 hours the worms were taken out and fixed, and sectioned to see if they had swallowed the spores and, if so, what was the effect on these. Unfortunately the experiment proved nothing except that spores containing apparently normal sporozoites do survive in the gut of worms that have been deliberately fed on such material. No sign of hatching has been seen. I have also placed ripe spores in the body fluid of worms and kept them under observation for long periods; but without result. At present I am repeating the experiment.

VII. PRELIMINARY NOTES ON SOME OTHER SPECIES OF *SELENIDIUM* FROM POLYCHAETES.

I think it well to record now some notes that I have made on some other gregarines (some of them hitherto unrecorded) from polychaetes. My observations are fragmentary, and all I propose to do at present is to give a brief description and figures of the adult trophozoites. So far as the general morphology goes and the method of association, there seems every reason to suppose that these gregarines from *Branchiomma vesiculosum* Mont., *Sabella pavonina*

[1] These observations were made on living material, as the fixed material did not give satisfactory results.

Sav., *Pomatoceros triqueter* L. and *Terebella lepidoria* Kühl. belong to the genus *Selenidium*, and the types that I shall describe are easily distinguishable. It is probable that they merit special classification, but until I know something further regarding their schizogony and sporogony I prefer to do nothing further than to record and place them provisionally in the genus *Selenidium*.

(*a*) *Selenidium plicatum* n.sp. (Pl. XXXVII, fig. 22.)

In 1899 Caullery and Mesnil described two species of *Selenidium* from *Cirratulus cirratus* Muell. and called one "en virgule" and the other "point et virgule" on account of a supposed resemblance in form to a "comma" and a "semicolon." Schizogony has not been observed yet in either of these and an early intracellular stage has only been seen in the second species. During my search for the stages in their sporogony, which was unfortunately disappointing, I came across a totally new form of gregarine in the gut of this worm. Out of fifty worms examined, only one showed this new form and I now propose to give a brief description of the trophozoite.

In my preparation there are individuals measuring between 220 and 380 μ in length and 24–30 μ in breadth. In the living condition they exhibit movement characteristic of the genus—that is, they move rhythmically from side to side, or twist or throw themselves into a spiral. I have not observed any movement of translation.

The nucleus is situated somewhat towards the anterior end, and its long axis is parallel to the transverse axis of the body; the poles of the nucleus do not usually reach the lateral margin of the body, as is usually the case with most of the other species of *Selenidium* described here. There is a spherical karyosome placed eccentrically. The anterior end of the body is slightly broader than the posterior and carries a knob-like epimerite with a darkly staining collar, which on careful examination appears to be composed of minute thread-like bodies arranged in a row. In the centre of the knob one or two rather darker, thread-like bodies can be distinguished. The body is not longitudinally striated but, instead, along the whole length of the body there are numerous Λ-shaped ridges running transversely and this gives a plicated appearance to the parasite, both in the living and in fixed preparations. The arms of the ridges converge along the whole of the median length of the body and thus give rise to a long dark median rib. The cytoplasm is hyaline throughout.

(*b*) *Selenidium* spp. from *Branchiomma vesiculosum* Mont.

I have examined about 50 *Branchiomma vesiculosum* from Plymouth and have invariably found the gut inhabited by gregarines, which, pending further investigation, I have placed in the genus *Selenidium*. The parasites are always located in the anterior half of the digestive tube (as in *Myxicola infundibulum*), and the largest trophozoites and the associating forms are actually to be found within the first few segments. Towards the middle of the

gut very young forms are found hanging by their modified anterior ends to the epithelial cells, which apparently they damage. Amongst these gregarines I have been able to distinguish two types, whose adult morphology only is described here.

I have not yet traced these beyond the stage of association (when they come together by their posterior ends), and, as I have said above, this stage occurs at the anterior end of the gut, which is perhaps not unnatural in a tube-dwelling worm. I presume that these forms are expelled into the sea-water by sudden retraction of the worm, and then proceed with development of the spores. Whether or not the further developmental history falls in line with that of the gregarines already described I cannot say. This is still under investigation and the results will be published later on.

(i) *Selenidium branchiommatis* n.sp. (Pl. XXXVIII, fig. 27.)

The worm-like adult individuals, which apparently are the gametocytes, when examined in sea-water execute movements characteristic of the genus; they bend to and fro, become S-shaped or twist spirally. The cuticle is marked by 25 to 35 longitudinal striations. The length of the trophozoites varies between 75 μ and 600 μ, while the breadth lies between 10 μ and 20 μ. At the anterior end there is a knob-like elevation which is used for attachment to the host cell. At the base of this knob there is a vacuole, which appears to be lined by a number of refringent strands (Pl. XXXVIII, figs. 27, 28) clearly visible in the living specimen. Sometimes one or two of these strands can be traced into the knob (Pl. XXXVIII, fig. 28). They stain black in iron haematoxylin and pink in Dobell's modification of Mann's methyl-blue-eosin. They seem, therefore, to be homologous with similar bodies in the species I have already described; their presence helps one to determine the anterior end even at a very young stage (Pl. XXXVIII, fig. 26). In young forms these strands are crowded more towards the nucleus and as the parasite grows they appear to be carried forwards to their final position.

The nucleus is oval, its long axis is parallel with that of the body of the gregarine and it is usually situated midway between the anterior and posterior ends of the body. The nuclear membrane is thick and conspicuous in the adult, but less so in the young forms. There is a large spherical karyosome (reaching a diameter of 10 μ in the full-grown parasite) situated towards the posterior end of the nucleus. Sometimes two to four vesicles can be detected within the karyosome.

(ii) *Selenidium alleni* n.sp. (Pl. XXXVII, fig. 23.)

This species is not so common in *Branchiomma* as is the last species, from which it can be readily distinguished by its smaller size, 12–120 $\mu \times$ 6–15 μ, by the small number of longitudinal striations (about 12 to 16) and by the fact that the oval nucleus has its long axis at right angles to that of the gregarine[1]. Moreover, the anterior end is pointed, and at its extremity there

[1] In the young forms the nucleus has its long axis in the direction of that of the previous species.

is a siderophilous ring from which a similarly staining strand runs into the endoplasm (Pl. XXXVIII, fig. 24); the posterior end of the body is rounded. The characteristic siderophilous structure can be detected even when the young gregarine measures only about $12 \times 6\mu$ (Pl. XXXVIII, fig. 25). The nuclear membrane is thin as compared with that of the previous species. The spherical karyosome lies towards the posterior end of the nucleus, but does not show any intrakaryosomic vesicle.

Association is effected by the posterior ends of the gametocytes, and the nucleus of each at this stage is drawn out into a transverse spindle within which a number of vesicles, seemingly the gamete nuclei, can be detected. The siderophilous structure at the anterior ends of the associates can still be seen.

(*c*) *SELENIDIUM SABELLAE* (LANK.) (Pl. XXXVIII, fig. 37.)

While I was collecting data on schizogregarines, my attention was drawn by Miss E. A. T. Nicol to a gregarine inhabiting the gut of *Sabella pavonina* Sav. Curiously enough, the infection here is confined to the posterior half of the digestive tube—which is contrary to what happens in *Myxicola infundibulum* and *Branchiomma vesiculosum*. The gregarine shows morphological features quite comparable with those of the parasites already described, so provisionally I place it in the genus *Selenidium*[1].

Gregarines of all sizes from $24\mu \times 8\mu$ to $120\mu \times 60\mu$ are found attached to the epithelial cells of the host by their curiously modified anterior ends (Pl. XXXVIII, fig. 39). There is a cone-like elevation which is pushed right into the host-cell and, when carefully separated therefrom, it appears to pull out the cytoplasm of the cell as protoplasmic strands which hang round the epimerite (Pl. XXXVIII, fig. 38). Within this cone there is a vacuole which contains four or six refringent thread-like bodies comparable with those in the other species of *Selenidium* described here. The body, which is circular in transverse section, is widest in the middle and gradually tapers towards either end; the posterior end is broader than the anterior end and becomes more so with age. In a full-grown form, apparently a gametocyte, the posterior end becomes very much broadened out and spatulate in shape—probably a modification prior to association.

When the gregarine is examined fresh and *in situ*, the posterior end shows a pendular movement, but when it is pulled out of the gut wall its anterior end appears to be more mobile than the rest of the body. Sometimes I have seen that the posterior end gets fixed to the slide, while the anterior end moves in all directions as if feeling for something. Here it differs from other selenidiids, where the posterior end takes an equal, if not greater, share in producing the characteristic movements. There are fine longitudinal striations on the ecto-

[1] Lankester (1863) described gregarines from the worms that he named *Sabella alveolata*, *S. bombyx* and *S. impendiculum*, and to him they all appeared to belong to one species. *Sabella pavonina* Sav., seems to be one of the synonyms for *Sabella alveolata* Linn., which fact further confirms me in the opinion that Lankester saw the parasites that I here describe.

plasm, which appear to increase in number with the growth of the parasite; for instance, in an individual measuring 60 μ long and 20 μ broad there are about 24 to 26 striations, while in a full-grown parasite there are as many as 40. There is a thin pellicle and the endoplasm stains evenly.

The nucleus is spherical and usually lies in the middle of the body. There is a spherical karyosome, usually lying towards the posterior end of the nucleus, and this, together with the thread-like bodies at the anterior end, stains pink with Mann's methyl-blue-eosin and black with iron haematoxylin. Before the gregarine has reached its gametocytic stage, its nucleus, except for the karyosome, shows scarcely any darkly staining granules; but as the parasite advances in age, these begin to appear in the nuclear substance, while the karyosome is correspondingly reduced.

Gametocytes apparently become detached from the host cell and associate by their posterior ends in the lumen of the gut. Unfortunately I have not seen them pass beyond this stage and therefore I am unable to throw any light on the process of spore formation. Though I have examined 50 worms, I have not yet come across the schizogony, intra- or extracellular, which is supposed to be the chief characteristic of schizogregarines.

(*d*) *Selenidium brasili* n.sp. (Pl. XXXVIII, fig. 34.)

I examined a large number of *Pomatoceros triqueter* at Plymouth with the idea of finding out the life-history of a gregarine with which the intestine of this worm is commonly infected. At present I can do no more than give the following data[1].

Young trophozoites, 30–40 μ long and 8–10 μ broad, are commonly found hanging on to the epithelium by their modified anterior ends. On being detached from their hold, their anterior ends exhibit a to-and-fro movement very similar to that of the gregarine from *Sabella* (see p. 389). The anterior end is broader than the posterior, and carries a knob-like epimerite at its extremity, which is sometimes torn off when one makes smears from the gut wall. The epimerite, as in other species, contains six to eight refringent thread-like structures (Pl. XXXVIII, fig. 35); young individuals, 30–50 $\mu \times$ 12–16 μ, appear to possess eight to ten such bodies (Pl. XXXVIII, fig. 36). Large individuals, apparently gametocytes, measuring about 150–180 $\mu \times$ 20–35 μ, are often found in smears. There are about 30 to 35 longitudinal striations on the body, which is approximately circular in cross-section. The nucleus is spherical and situated slightly toward the anterior end. There is a spherical karyosome, which is usually placed at the posterior pole of the nucleus. Sometimes one can see a cap-like structure at the anterior pole of the nucleus (Pl. XXXVIII, figs. 34, 36). Association takes place by the posterior ends, which have now become very much broadened. The nuclei of the gametocytes appear to behave in the same way as those of *S. caulleryi*, *S. spionis*, etc. (see pp. 376, 384).

[1] Caullery and Mesnil (1899) record a specimen of *Selenidium* from this worm, but unfortunately give no description or figures of it.

(*e*) *SELENIDIUM TEREBELLAE* (Köll.). (Pl. XXXVIII, fig. 29.)
(= *ESARABDINA TEREBELLAE* Mingazzini.)

In the month of September, 1929, I examined some specimens of *Terebella lepidoria* Kühl at Plymouth. The middle and posterior regions of the gut of the worms were found to be infected with a gregarine which seems to correspond with what Kölliker (1845 and 1849) described as *Gregarina terebellae* from the gut of an unknown species of *Terebella* from Naples. Kölliker's account of the parasite is very slight: Mingazzini (1891) makes some brief mention of this parasite, which he calls *Esarabdina terebellae*. I therefore propose to give some further notes which indicate that it is morphologically comparable with the other species of *Selenidium* described here.

In the living condition, the parasite executes the movements characteristic of the genus; the anterior end is more mobile than the posterior and turns in all directions; sometimes the body twists round its own long axis. It is probably this movement to which Kölliker refers when he says, "Die Bewegungen dieser Art sind ziemlich lebhaft, schlängelnd." The twisting sometimes goes so far that, at first sight, the pellicle seems to be obliquely striate; but if watched carefully for a while, the organism unwinds itself and shows only six to seven longitudinal striations. It should be noted here that young stages, 30–50 $\mu \times$ 12–15 μ do not show this kind of movement and have only three or four longitudinal striations.

The body is spindle-shaped and is broadest in the region of the nucleus, which is situated somewhat behind the middle. The anterior extremity shows a cone-shaped elevation within which a vacuole containing a number of refringent thread-like bodies can be distinguished in the living.

In my preparation I have come across individuals of varying dimensions, 30–300 $\mu \times$ 12–28 μ (Pl. XXXVIII, figs. 31, 32). The parasite is circular in transverse section and shows a definite pellicle, below which lie six or seven rib-like projections into the endoplasm (Pl. XXXVIII, fig. 30), which is uniformly granular.

The nucleus is oval, with its long axis lying at right angles to the long axis of the body, the nuclear membrane is well marked and between it and the nuclear substance a clear area traversed by a few strands can always be distinguished; there is a spherical karyosome lying towards one end in the nucleus.

I have not seen this organism preparing for sporogony, nor have I seen schizogony, either extra- or intracellular.

VIII. DISCUSSION.

(*a*) SCHIZOGONY.

The Schizogregarinida are distinguished from other acephaline gregarines by having an asexual schizogonic multiplicative phase in their life cycle. Mesnil (1899) held that they form a heterogeneous and artificial group, and he declared that certain genera now placed therein would one day be transferred to the Eugregarinida. Léger in 1910, however, considered the schizogregarines

to be a homogeneous group, monophyletic in origin. Keilin (1923) reverted to Mesnil's opinion, and I feel disposed to agree with him.

There seems no doubt that in certain families schizogony does normally and regularly occur. The schizogregarines of insects have received a good deal of attention from protozoologists, and the behaviour of the various species of *Ophryocystis*, for example, is sufficiently stereotyped to make the occurrence of schizogony a feature of diagnostic value. But our knowledge of some of the other genera is in a much less satisfactory state. Protozoologists finding an acephaline gregarine in the gut of some invertebrate have been too ready to conclude that they were dealing with a schizogregarine, and have at once looked for its asexual multiplicative stage; with this prepossession in their minds, they have been misled by appearances that seemed susceptible of such interpretation, but in reality had nothing to do with the parasite they were immediately studying. Such is probably the explanation of the endogenous budding described by Dogiel in *Schizocystis sipunculi* from *Sipunculus nudus*. And such, I now maintain, was the mistake made by Caullery and Mesnil when they were examining *Scolelepis fuliginosa*, and tacked on to the life history of the "schizogregarines" with which they were concerned the schizogony of another sporozoan, which I believe to be a coccidian.

The genus *Selenidium* is one of the least satisfactory in all the group. Its present definition rests, as I consider, on a piece of faulty observation. Intracellular schizogony does *not* normally occur in the two species of *Selenidium* from *Scolelepis*, if the results of the laborious examination of some 5000 slides of 250 infected worms may be taken as evidence. Neither does extracellular schizogony occur. So it would seem at present that these two gregarines are neither species of *Selenidium* nor are they schizogregarines in the accepted sense. It may be, of course, that they go through a schizogony of some sort while within the body of some unknown second host, but I think this very improbable.

So far as I have studied them, the "schizogregarines" morphologically indistinguishable from *Selenidium* occurring in *Branchiomma*, *Cirratulus*, *Sabella*, *Pomatoceros* and *Terebella* are likewise guiltless of schizogony; but I do not wish to be dogmatic on this point, as my search in these worms has not yet been sufficiently exhaustive.

Brasil's description, accompanied this time by figures, of schizogony in the *Selenidium* from *Protula*, seemed to conform with the generic definition. I had become rather sceptical about the intracellular schizogony of *Selenidium*, and I thought that there might be some snag here also. But though I have had only a small amount of material I have confirmed his observation that, in *S. caulleryi* an intracellular schizogony *does* occur (see Text-figs. 1 and 2).

I find that the behaviour of *S. mesnili* agrees with that of *S. caulleryi*. Here also there is a definite intracellular schizogony, of the same type as in Brasil's first species, and this is also, therefore, a true *Selenidium* according to his definition.

The conflicting evidence leads one to wonder, then, whether the occurrence of schizogony is really an invariable feature of this group. I have for the present left the parasites of *Scolelepis* with the old generic name, but I am coming to the conclusion that the genus *Selenidium* as defined by Brasil is not really homogeneous at all. Strictly speaking, the type species of the genus is *Selenidium pendula* Giard. This is said to occur in the polychaete *Nerine*, but the species of *Nerine* was not stated by Giard. It is obviously of importance to discover what are the characteristics of *S. pendula* in respect of schizogony and the contents of its spore, for, until this is done, any attempt to dismember the genus *Selenidium* may only lead to further confusion. I have examined the gut contents of four specimens of *Nerine foliosa* Aud. and Edw.[1] at Plymouth, but, so far, I have failed to find any gregarines in it. I am inclined to believe that Giard was dealing with another species, but I shall carry my investigation further. The whole matter wants much further study and on an extensive material. I hope to be able to make some more definite statement at a future time.

(*b*) THE "CHROMATIC" BODIES.

If intracellular schizogony is *not* an invariable feature of the gregarines grouped together in the genus *Selenidium*, then, in considering new species, more attention must be paid to the morphological characters of the adult trophozoites and to characteristic features in their sporogony. If, on the other hand, the occurrence of an intracellular schizogony *is* to be the test, then certain common morphological features and modes of behaviour must be disregarded, characteristics which would naturally incline one to place organisms like the gregarines from *Protula* and those from *Scolelepis* in the same genus.

I have shown that, in all the gregarines considered in this paper, and at all stages of these that I have seen, there lie at the anterior end of the body certain refringent structures which are very characteristic. These are usually thread-like, sometimes club-shaped, but always of a definite type and length in any one species, and usually pretty constant in number. They are easily seen in the living animals, and I cannot understand how they have been so seldom noticed before. Caullery and Mesnil have noted a dark-staining area at the anterior end of *Selenidium* "en virgule" from *Cirratulus cirratus*, but Brasil is the only author who makes any exact reference to the structures; he shows them very clearly in his figures of *S. mesnili*, but only in one figure of *S. caulleryi*, and in that species he saw them only now and then vaguely in the half-grown forms, and he seems to have missed their value from the observer's point of view. For by their aid it is quite easy, in well-stained and well-differentiated preparations, to pick out the gregarines from other parasites; and this is important when one is dealing with a mixed infection.

What the function of these refringent bodies is I do not know, nor do I know their provenance. I have already noted that their staining reactions are similar to those of the "karyosome" in the nucleus. In *S. mesnili* and

[1] Two species, *Nerine foliosa* and *N. cirratulus* are available at Plymouth, but are very rare.

S. caulleryi they do not appear to multiply when schizogony comes on, and in all species they seem to fade away after association of the gametocytes, though they reappear once more in the merozoites, and also in the sporozoites of those two species of which I have obtained the spores. I get the impression that they are products of the nucleus, but I am not yet confident about this. Similar bodies have been noticed in some Coccidia, in *Angeiocystis audouiniae*, for instance; and in a later paper I shall show that they also are found invariably in the coccidian from *Scolelepis* (though there they are of quite a different form from the fibrils in the accompanying gregarines). Perhaps the siderophilous epimerite of gregarines like *Monocystis* and *Lankesteria* is of the same nature.

The swaying movements, witness to powerful muscular differentiation in the ectoplasm, are found in all the forms I have described and are generally held to be typical of these schizogregarines.

(*c*) The intracellular period.

It is evident from what I have observed that the species of *Selenidium* behave very differently from one another in respect of the length of time they pass within the epithelium of their worm host.

S. mesnili is intracellular until the gametocytes, already showing some nuclear preparation for association, fall out into the lumen of the gut. *S. caulleryi* is intracellular for a much shorter time; those trophozoites that are going to become gametocytes escape into the gut when they are about 15–20 μ long and there they continue to grow to 60–75 μ or more. *S. foliatum* is intracellular for a brief period only: it leaves the host cell when it is about 30–35 μ in length, and though it may remain attached thereto by its anterior end, it passes nearly all its life in the lumen, where it grows to a length of 250 μ before proceeding to association. Finally, *S. spionis* seems scarcely ever to be intracellular at all, except possibly just in the very beginnings of growth, and the very occasional inclusion of a large individual below the epithelium is probably an abnormal and accidental thing.

It is possible that the development of schizogony is, in some way, definitely associated with increase in the intracellular habit. If that is the case, then creatures like the selenidiids from *Scolelepis* may be regarded as the more old-fashioned and the less specialised in their behaviour.

(*d*) Sporogony.

Very little has been noted by earlier observers regarding sporogony in these parasites. The gametocytes of *S. echinatum* and of *Selenidium* sp. No. 1 (now called *S. spionis* (Köll.)) were seen by Caullery and Mesnil in association in the gut. These authors say that attachment is made by the anterior ends. In the species I have examined, I always found that the associates become attached by their posterior ends, and, as Caullery and Mesnil did not observe the chromatic bodies in their gregarines which give one the sure clue, I think it very likely that they may have been mistaken.

Spores have only once been seen before—in *S. echinatum*. This is a form that I have unfortunately not had opportunity to examine, but the sporocyst is said to be roughened and to contain four sporozoites. That spore-formation should hitherto have escaped most observers is not surprising; now that I know how to obtain it, I hope to get some more certain information as to the number of sporozoites the spores usually contain. The number of sporozoites in a spore is generally considered a character of some importance in determining the affinities of Sporozoa within a group. There can be no question that the two species of *Selenidium* in *Scolelepis* have a different number of sporozoites—the spores of the one have four, as in *S. echinatum*, but those of the other have eight. This is rather surprising, and I intend to make a careful study of similar stages in other species, for the indications are that on this ground, even if on no other, the genus may have to be subdivided.

Until a good deal more exact information on these points has been gathered —and to work on these gregarines is a very laborious undertaking—it is obviously impossible to say anything further with confidence. But I hope I have indicated enough to show that some revision of the genus *Selenidium* Giard as re-defined by Brasil is urgently required.

IX. SUMMARY.

1. The main facts in Brasil's (1907) account of the intracellular schizogony in *Selenidium caulleryi* from *Protula tubularia* have been confirmed. Early stages in the sporogony of this species are described now for the first time.

2. Intracellular schizogony is described for the first time in *Selenidium mesnili* Brasil from *Myxicola infundibulum*, and here also the early stages in sporogony are noted for the first time.

3. The life histories of Caullery and Mesnil's two unnamed species of *Selenidium* (now called *S. spionis* (Köll.) and *S. foliatum* n.sp.) from *Scolelepis fuliginosa* have been exhaustively studied. No schizogonic phase has been observed in either. The gametocysts, here described for the first time, are found to develop fully only after escape into the sea. The gametocysts of *S. spionis* are ovoid, $108\mu \times 60\mu$, and contain spores with four sporozoites: those of *S. foliatum* are spherical, 70μ in diameter, and their spores contain eight sporozoites.

4. New or hitherto little known species of *Selenidium* are recorded from *Cirratulus cirratus* Müll., *Branchiomma vesiculosum* Mont., *Sabella pavonina* Sav., *Pomatoceros triqueter* L. and *Terebella lepidoria* Kühl, and the morphological characters of the trophozoites are described.

5. A discussion follows of the value of the diagnostic characters of the genus *Selenidium*. It is suggested that this genus requires drastic revision and will probably have to be dismembered. Stress is laid, however, on the occurrence in all the gregarines examined, and at all the observed stages of their development, of characteristic chromatic bodies at the anterior end, structures which have hitherto escaped the notice of most observers.

I should like to thank Prof. Doris L. Mackinnon, who suggested this work and under whose direction it has been carried on. I am greatly indebted to her for all her expert help and criticism. My thanks are due to Mr Clifford Dobell, F.R.S., for valuable suggestions that he has made from time to time, and to Dr E. J. Allen, F.R.S., and other members of the staff of the Marine Biological Association at Plymouth for the kindness and hospitality which they have shown to me during my visits to their Laboratory. I am grateful to Mr C. Biddolph and Mr D. A. Kempson for rendering valuable aid in the laboratory at King's College. I should also like to acknowledge a grant-in-aid received from the High Commissioner for India.

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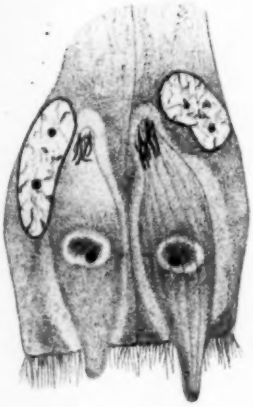

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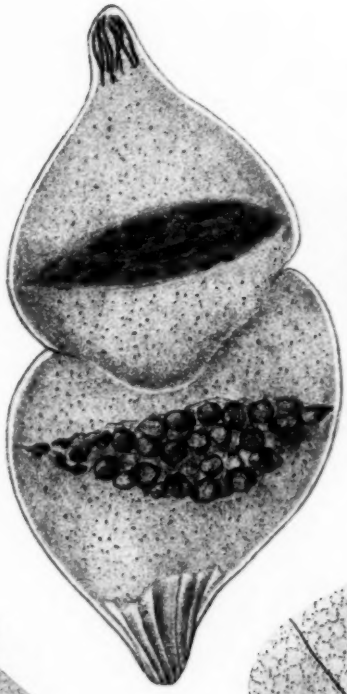

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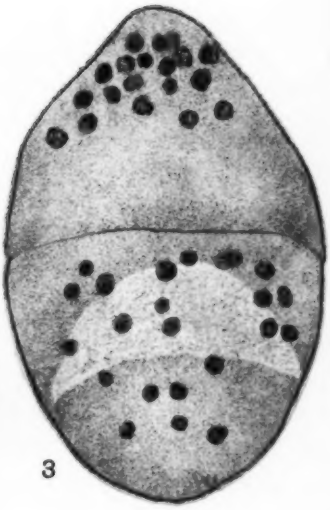

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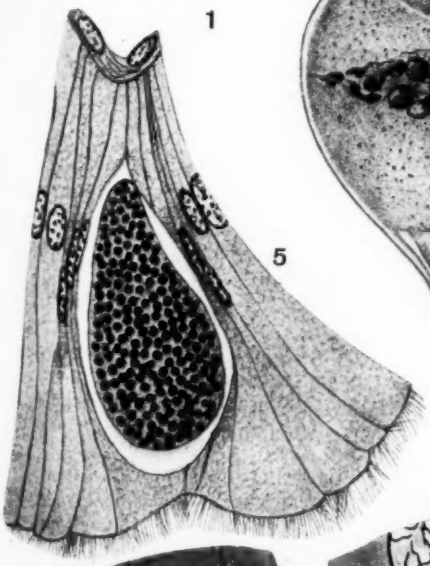

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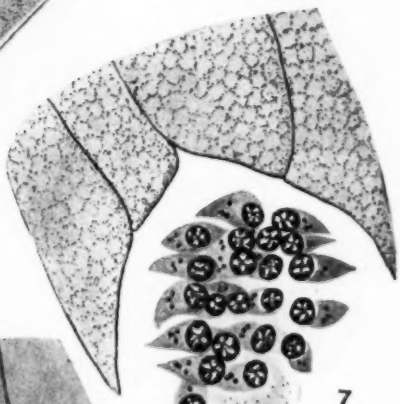

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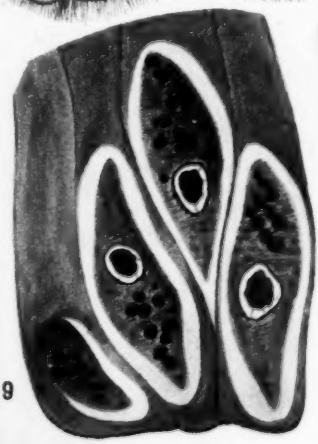

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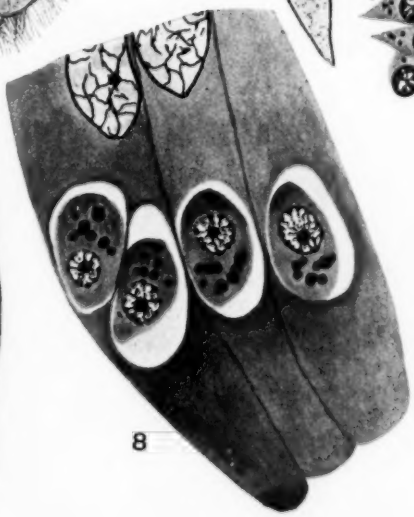

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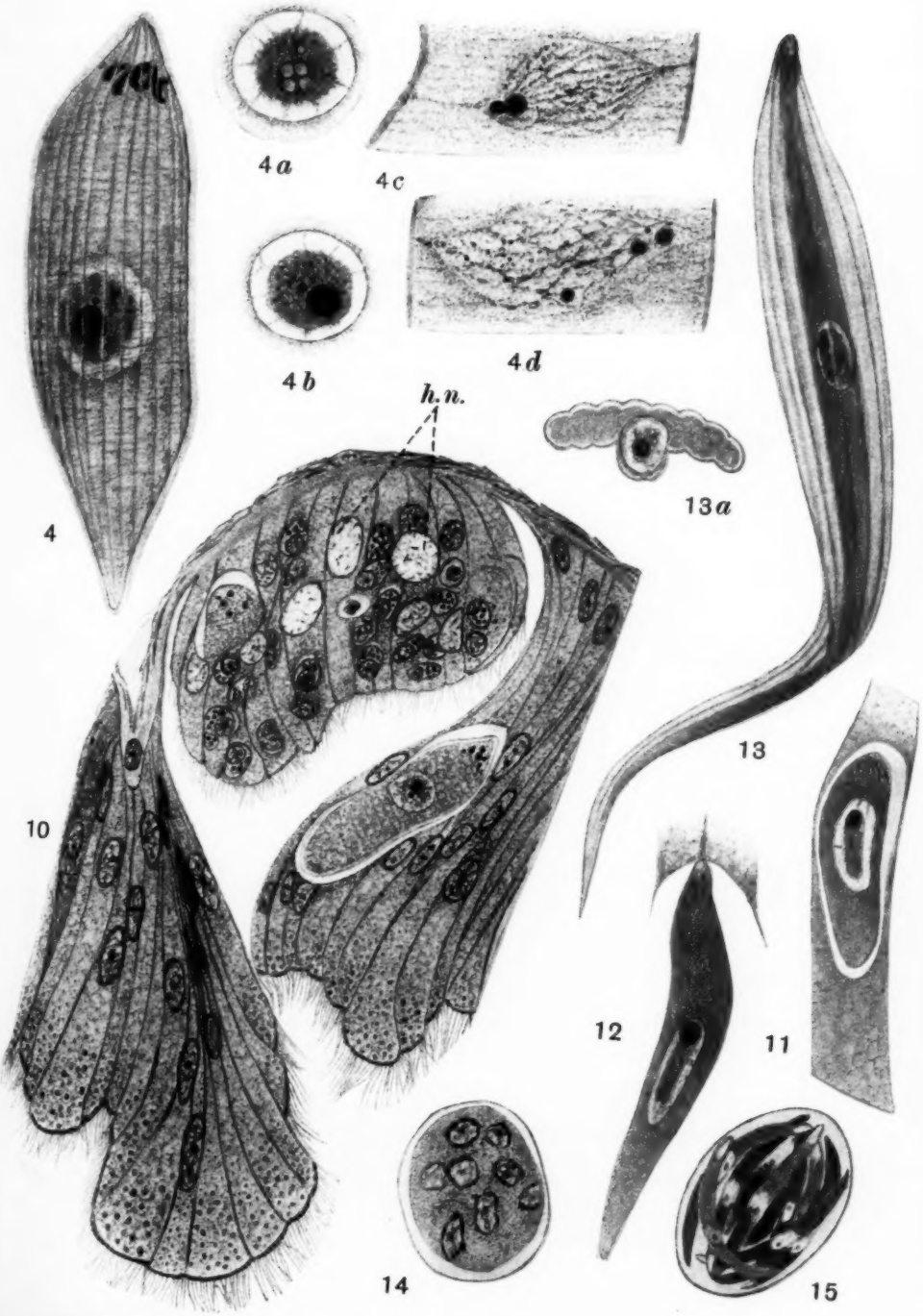

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DESCRIPTION OF PLATES XXXV—XXXVIII.

All figures are drawn with the aid of the *camera lucida* and the magnification is ×2100 unless otherwise stated. All drawings are made from smears or sections of species of *Selenidium* in polychaetes. The material was fixed in modified Bouin's fluid and stained in iron haematoxylin unless otherwise stated.

PLATE XXXV.

Fig. 1. *Selenidium caulleryi* Brasil. Young trophozoites. Note the thread-like structures at their anterior ends. (Longitudinal striations are only shown on one.)

Fig. 2. *S. caulleryi*. Association. The intracytoplasmic thread-like structures are shown only in one of the pairs, while in the other the longitudinal striations are indicated. Note small spindle-like bodies within one and vesicular bodies, the gamete nuclei in the other.

Fig. 3. *S. caulleryi*. Advanced gametocyst. Note the outline of a parent nucleus which is still persisting. Reconstructed from four sections.

Fig. 5. *S. mesnili*. A schizont, with numerous nuclei. Drawn from a section of the intestine of *Myxicola infundibulum*. (×500.) (Cilia of the epithelial cells omitted.)

Fig. 6. *S. mesnili*. A portion of the same to show the peripheral arrangement of the chromatin and the chromatic dots accompanying each nucleus.

Fig. 7. *S. mesnili*. A few merozoites. Note the dividing chromatic dots. (×4000.)

Fig. 8. *S. mesnili*. Merozoites after invading fresh epithelial cells. Note the increase in the number of chromatic dots, and the development of karyosomes within their nuclei. (Cilia omitted.)

Fig. 9. *S. mesnili*. Trophozoites. Note further increase in the number of the chromatic dots and the "zebroid" nature of their cytoplasm. (Cilia omitted.)

PLATE XXXVI.

Fig. 4. *S. mesnili* Brasil. A full-grown trophozoite. Note the club-shaped intracytoplasmic bodies at its anterior end. Drawn from a smear fixed in Schaudinn's fluid.

Fig. 4 *a*. *S. mesnili*. Nucleus of a specimen stained in Dobell's modification of Mann's methyl-blue-eosin, showing intrakaryosomic spherules. *b*. Nucleus of a specimen stained in iron-haematoxylin. *c*, *d*. Nuclear phases preparatory to division.

Fig. 10. *S. mesnili*. Section of the intestinal epithelium of *Myxicola infundibulum*. Note the hypertrophied nuclei (*h.n.*) of the invaded tissue which has shrunk away from the general surface to form a "peninsula." (×500.)

Fig. 11. *S. foliatum* n.sp. Young intracellular stage. Note the chromatic threads at the anterior end, which is directed towards the base of the epithelial cell.

Fig. 12. *S. foliatum*. Young trophozoite attached by its anterior end to the epithelium.

(Both Figs. 11 and 12 are drawn from sections of *Scolelepis fuliginosa* stained in Dobell's modification of Mann's methyl-blue-eosin.)

Fig. 13. *S. foliatum*. A full-grown trophozoite. Drawn from a smear. (×1000.)

Fig. 13 *a*. *S. foliatum*. T.S. in the region of the nucleus.

Fig. 14. *S. foliatum*. A sporoblast showing eight nuclei. Drawn from a section of a gametocyst, mounted in Euparal.

Fig. 15. *S. foliatum*. A fully developed spore. Note the residual mass of protoplasm in its centre. Drawn from living material.

PLATE XXXVII.

Fig. 16. *Selenidium spionis* (Kölliker). Young trophozoite. Drawn from a smear.
Fig. 17. *S. spionis*. A full-grown trophozoite. Note the median dark staining streak.
Fig. 18. *S. spionis*. T.S. in the region of the nucleus. Note the membrane round the nucleus and the fibrillar structure running towards the edges.
Fig. 19. *S. spionis*. T.S. in the post-nuclear region. Note the median canal and the lateral fibrillae.
Fig. 20. *S. spionis*. Spore with four nuclei. Drawn from a section of a gametocyst, mounted in euparal.
Fig. 21. *S. spionis*. A fully formed spore, with four sporozoites. Note the residual mass of protoplasm and the refringent area at one of the ends of the sporozoites. Drawn from living material.
Fig. 22. *S. plicatum* n.sp. Trophozoite. Drawn from a smear. (× 1000.)
Fig. 23. *S. alleni* n.sp. Trophozoite. (× 1000.)
Fig. 24. *S. alleni*. Anterior end of trophozoite. (× 2100.)
Fig. 25. *S. alleni*. Young trophozoites. Drawn from a section of the intestine of *Branchiomma*.

PLATE XXXVIII.

Fig. 26. *S. branchiommatis* n.sp. Young trophozoite. Drawn from smear.
Fig. 27. *S. branchiommatis*. Trophozoite. Drawn from smear. (× 1000.)
Fig. 28. *S. branchiommatis*. Anterior end magnified to show the chromatic thread-like bodies.
Fig. 29. *S. terebellae* (Köll.). Trophozoite. Drawn from smear. (× 1000.)
Fig. 30. *S. terebellae*. T.S. to show the arrangement of myonemes.
Fig. 31. *S. terebellae*. Young trophozoite showing the thread-like structures. Drawn from a smear.
Fig. 32. *S. terebellae*. Half-grown trophozoite. (× 1000.)
Fig. 33. *S. spionis*. Imbedded between the cells of the epithelium of the gut of *Scolelepis fuliginosa*.
Fig. 34. *S. brasili* n.sp. Trophozoite. Drawn from a smear. (× 1000.)
Fig. 35. *S. brasili*. Anterior end, showing the thread-like structures.
Fig. 36. *S. brasili*. Young trophozoite.
Fig. 37. *S. sabellae* (Lank.). Trophozoite. Drawn from a smear. Note the strands of cytoplasm of the host cell adhering to the epimerite.
Fig. 38. *S. sabellae*. Anterior end of the same, showing the dark staining thread-like bodies in the epimerite.
Fig. 39. *S. sabellae*. Young trophozoite, showing its mode of attachment to the host cell. Drawn from a section. (Cilia omitted.)

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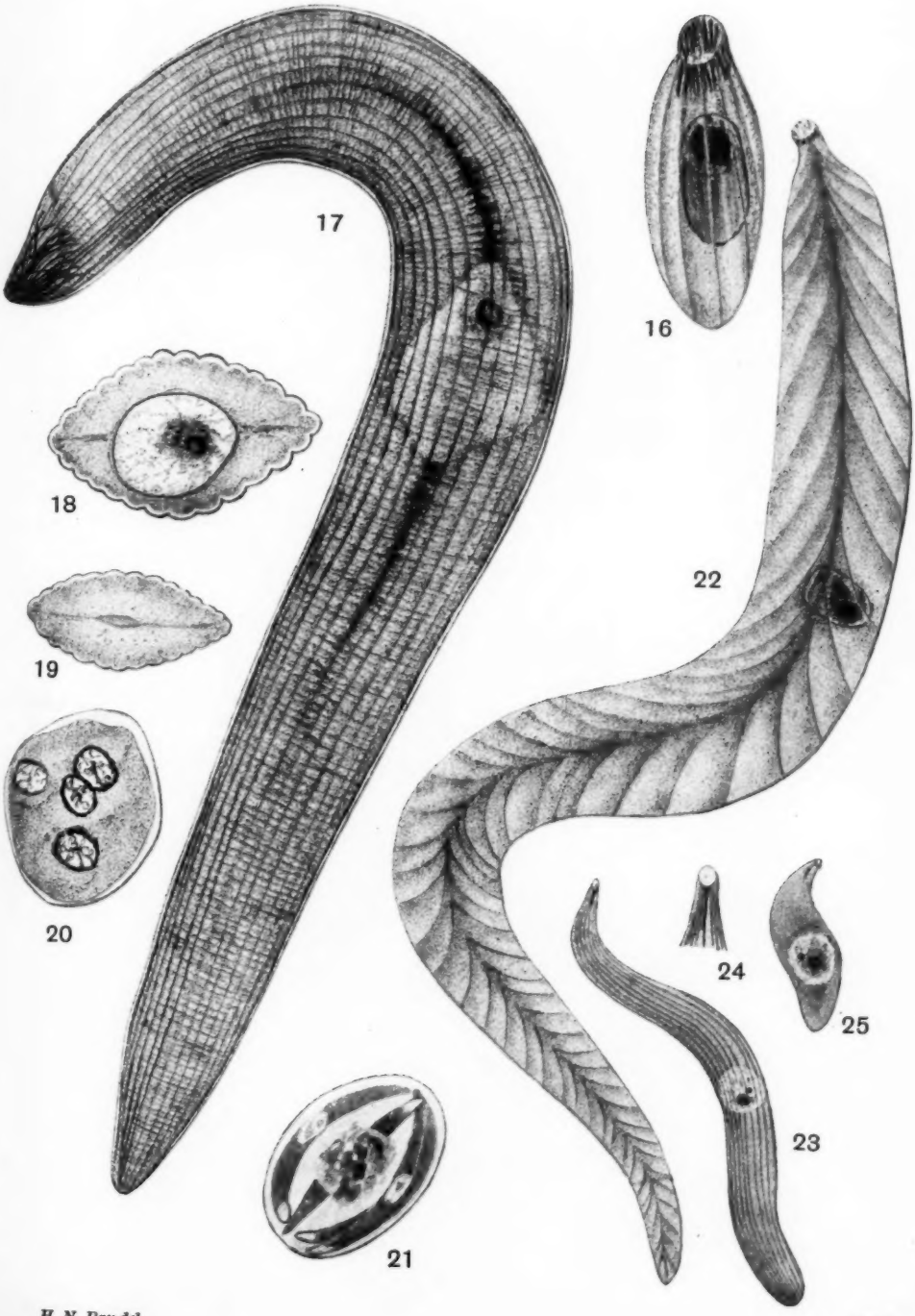

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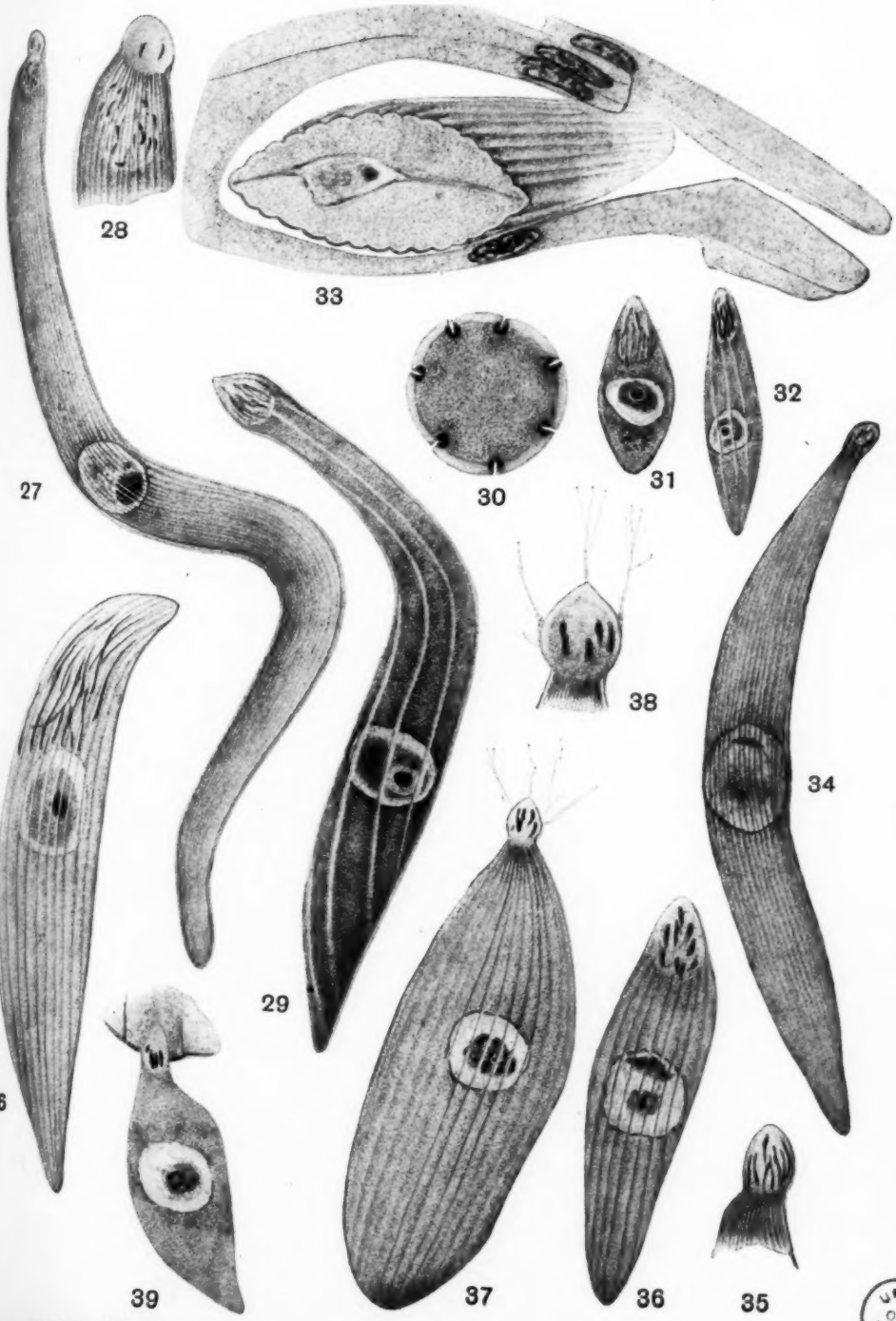

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